LABORATORY PRACTICE

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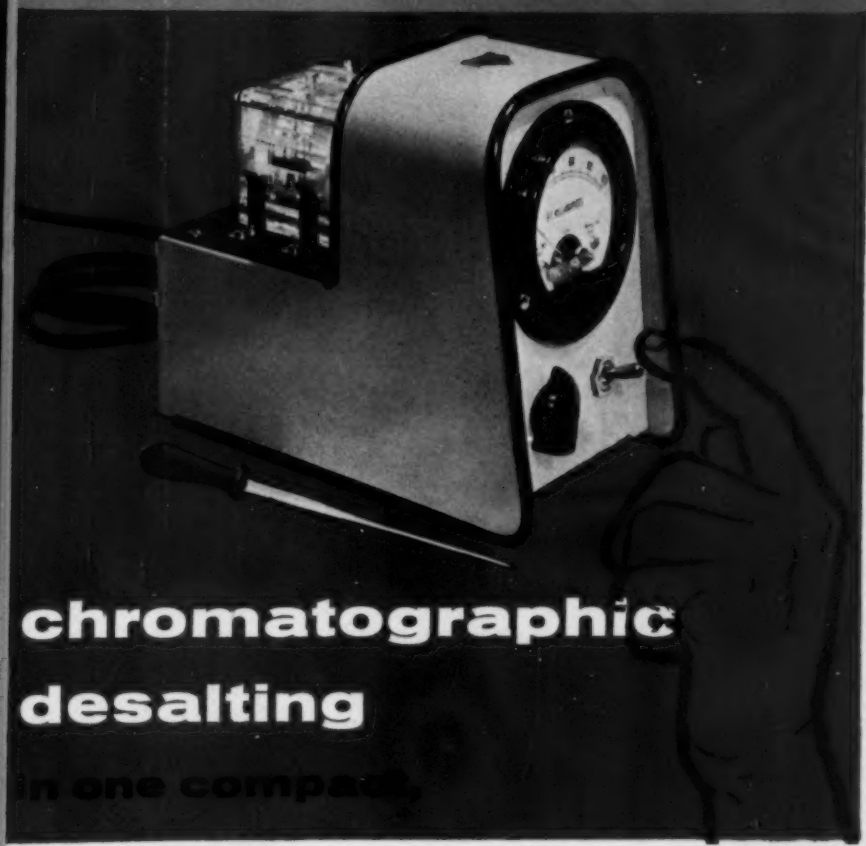

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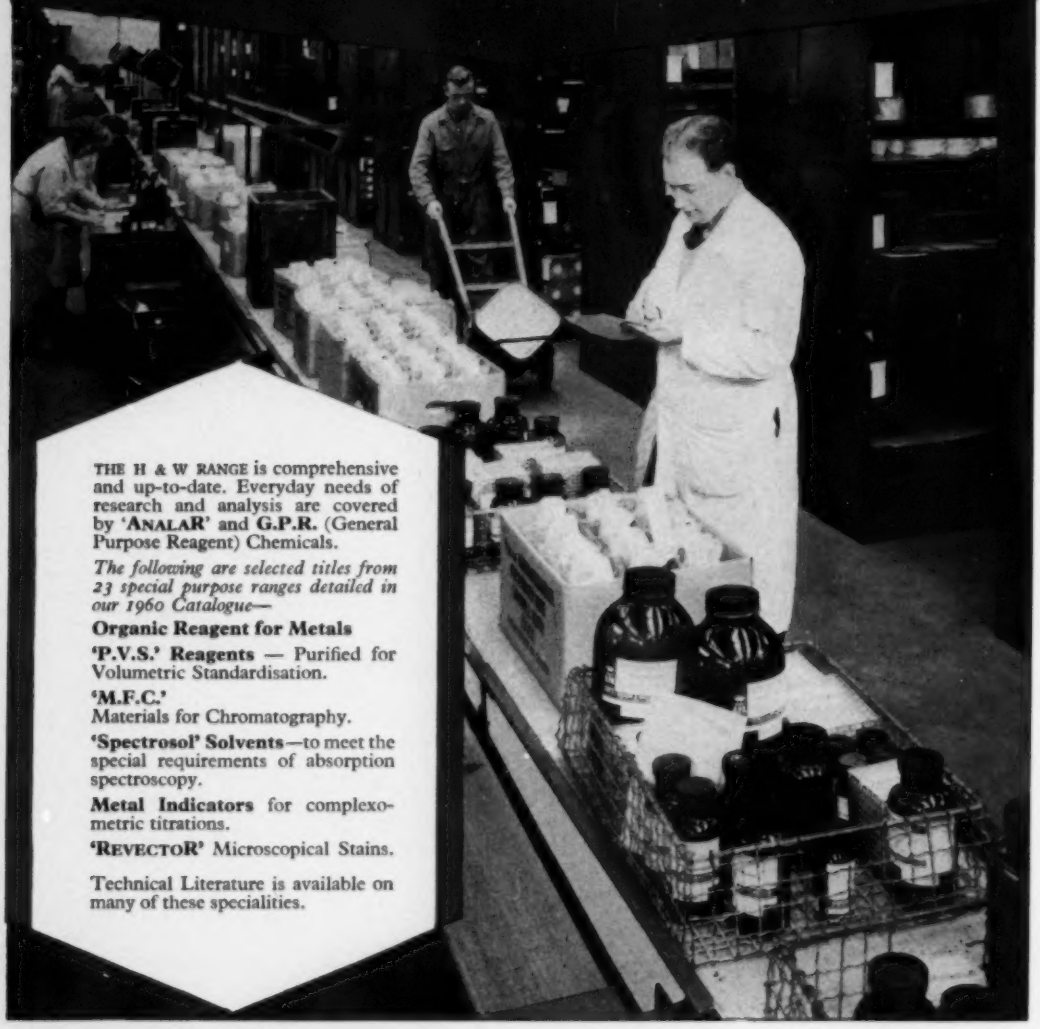

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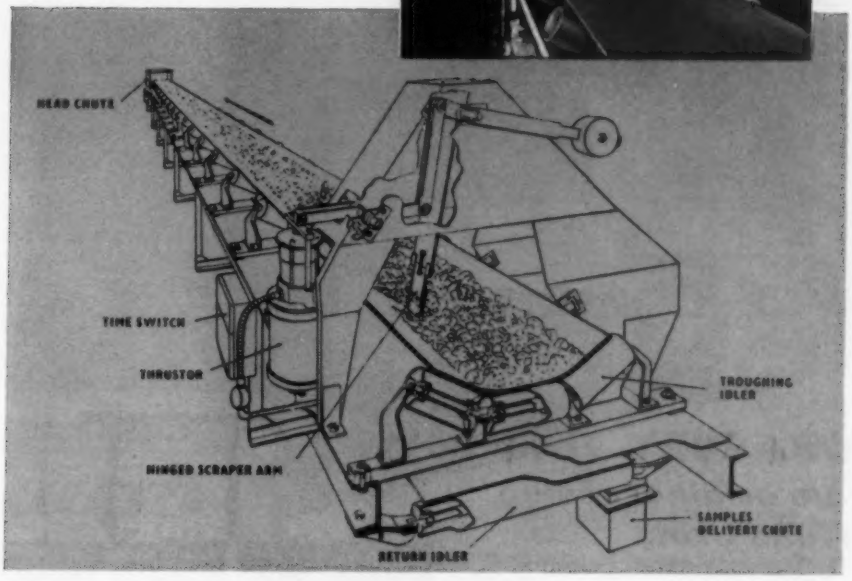

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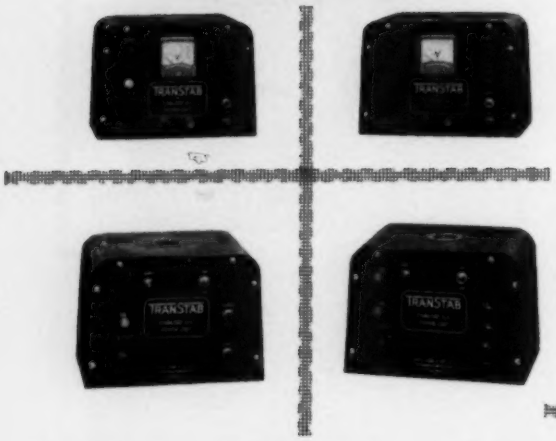

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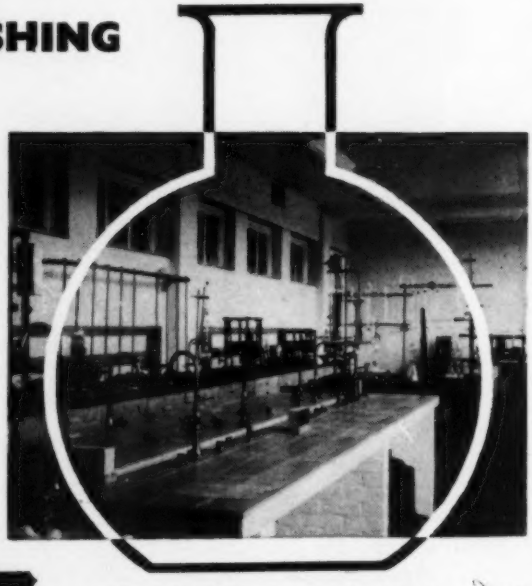

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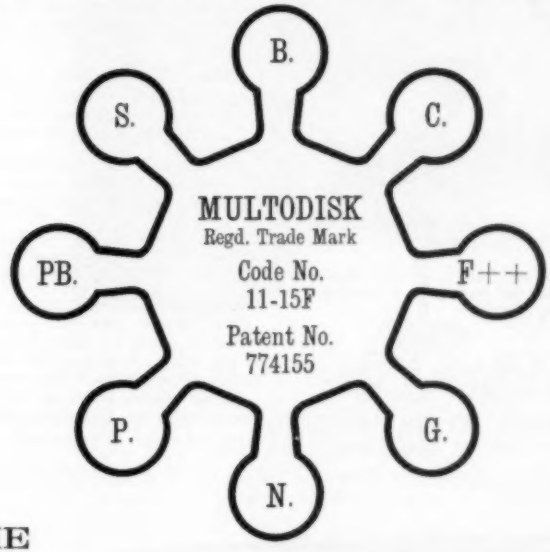

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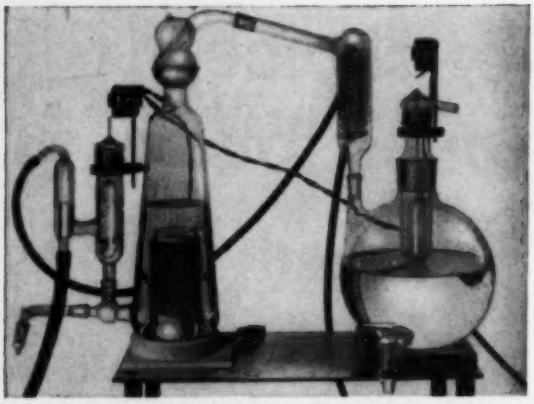

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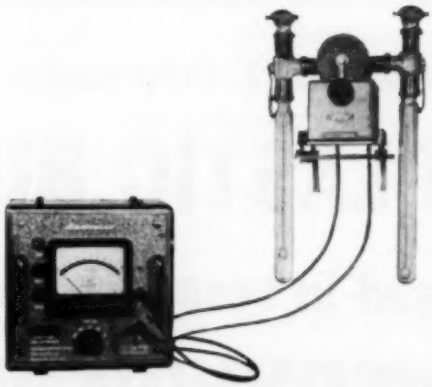

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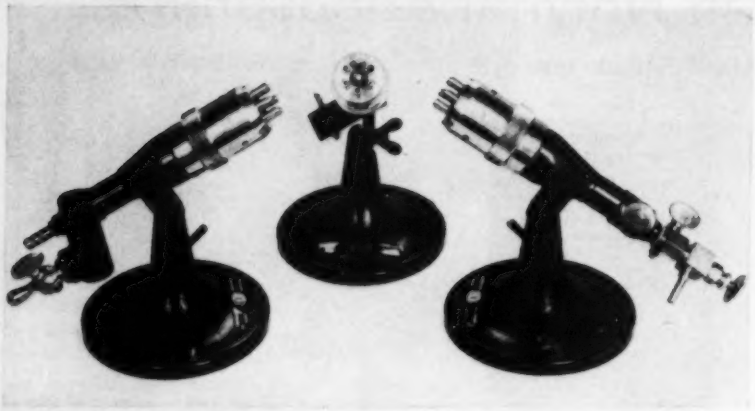

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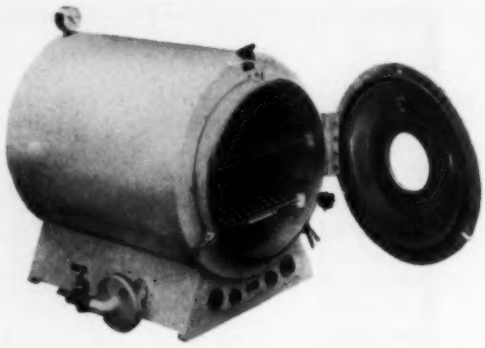

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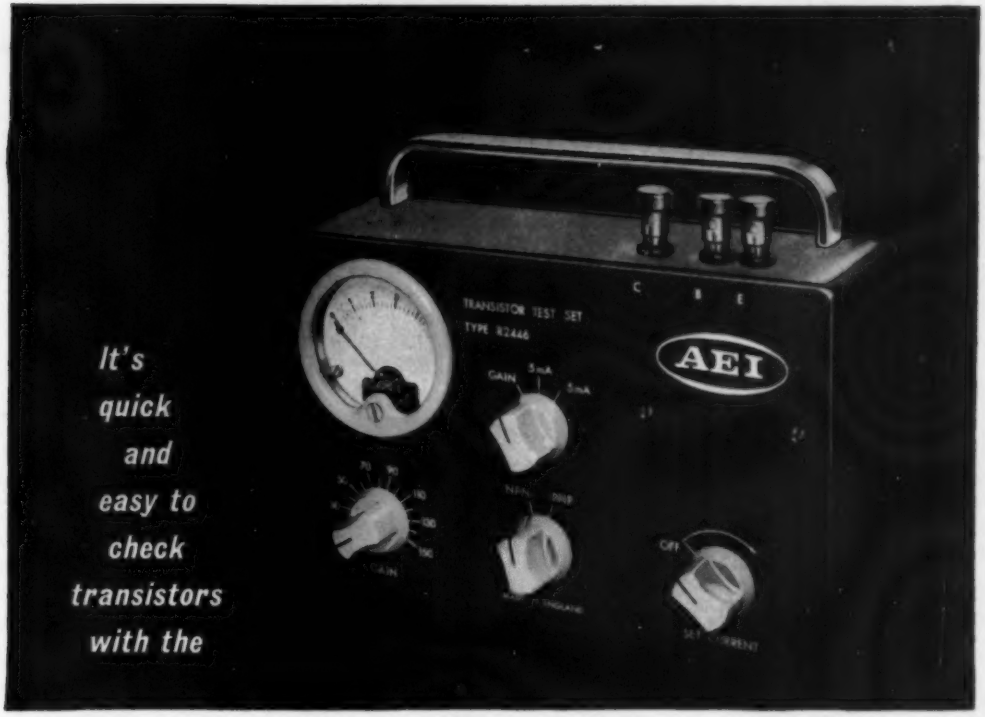

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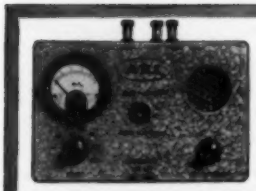

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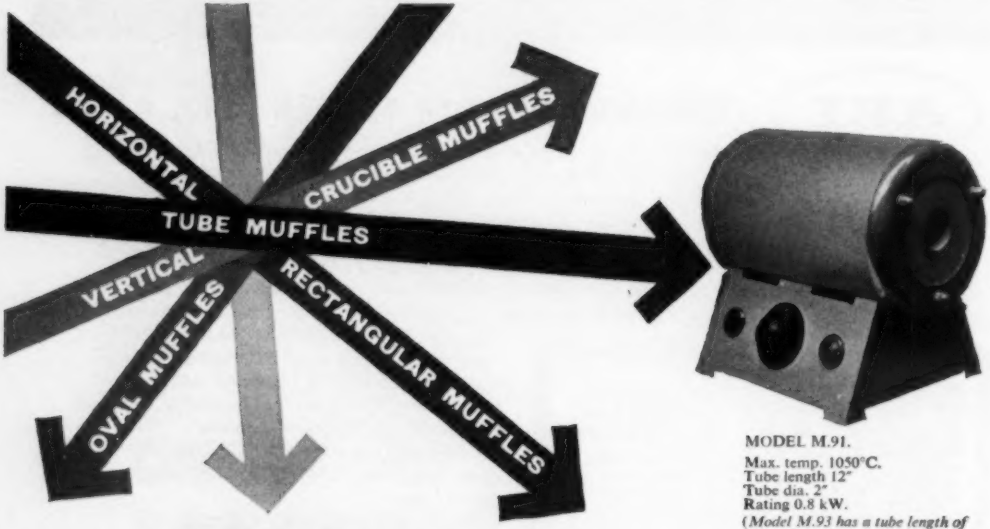

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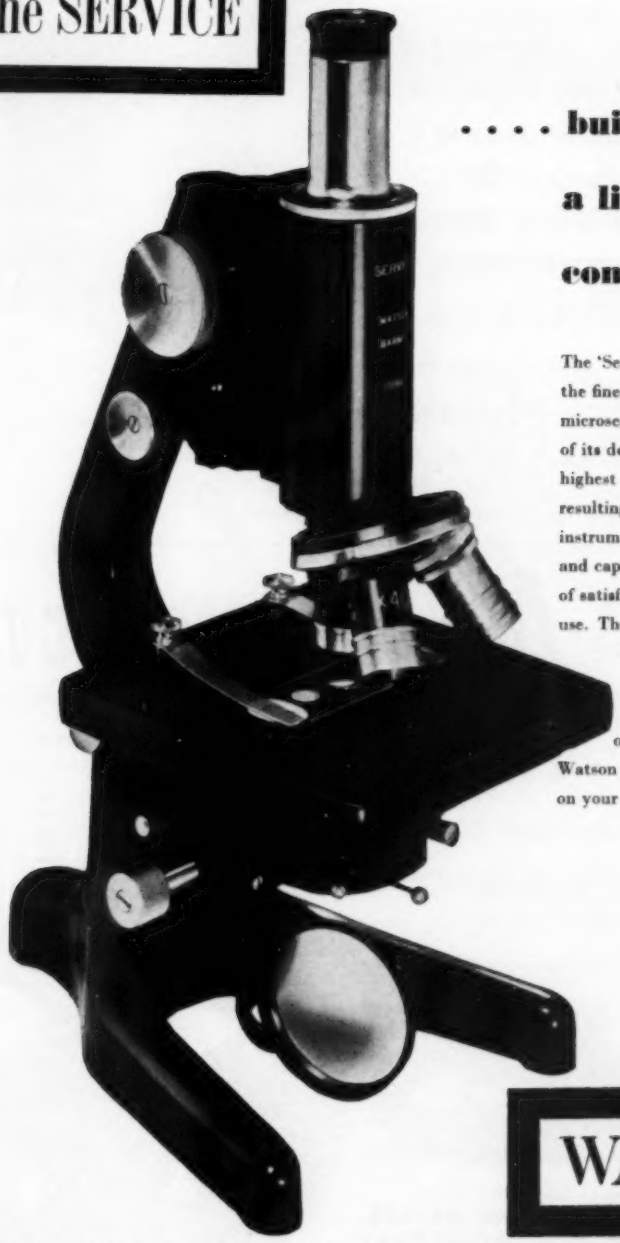

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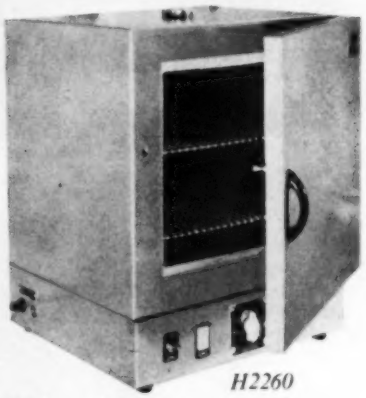

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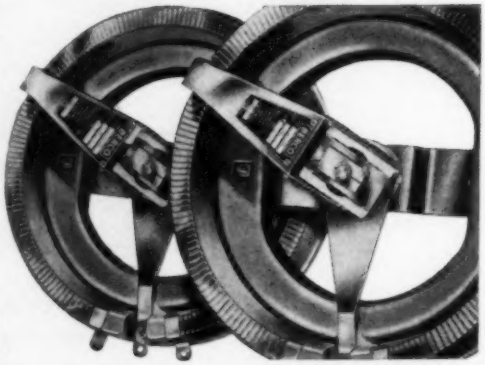

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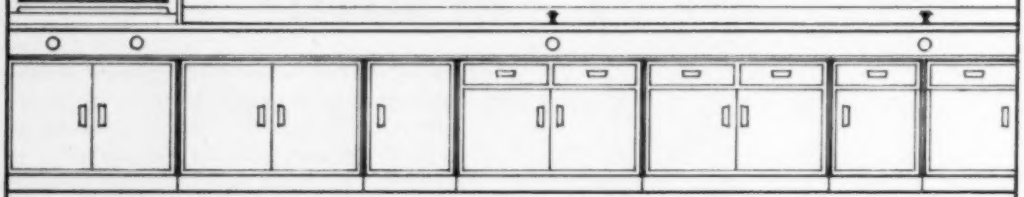

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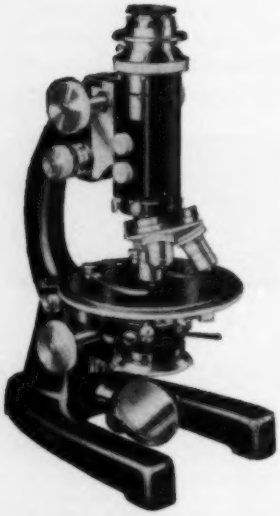

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EDITORIAL

The 1961 Laboratory Apparatus Exhibition

THE second Laboratory Apparatus Exhibition sponsored by 'Laboratory Practice' was again a complete success and followed very closely upon the lines of the first exhibition held last year. The very fact that a further exhibition was required so soon after the first demonstrates beyond doubt that there is a need for an exhibition of this type, in which all types and grades of laboratory workers can come to one central place in London and examine personally the latest developments in laboratory equipment, apparatus and materials. The stands again showed the high quality and imagination in the way in which they set out their apparatus, and the staff were equally able and willing to help enquirers on all subjects.

As last year, a series of lectures on recent advances in laboratory techniques was given by a distinguished panel of lecturers under the auspices of the Scientific Advisory Committee of 'Laboratory Practice'. The Exhibition was fortunate in having for their lecturers persons not only distinguished in their field, but with the greatest possible practical experience of the subject on which they were lecturing. As last year, these lectures were an unqualified success and all were attended by large and appreciative audiences. In fact some of the lecturers had difficulty in getting away from persistent questioners to enjoy some well-deserved refreshment.

Whether scientists came to the Exhibition in order to hear the lectures or whether they came to the lectures in order to see the Exhibition must remain a matter of opinion, but there is no doubt that these two activities together constituted a focal point of the greatest interest to all types of scientists.

The organizers of the lectures had gone to some trouble in order to cover the widest possible range of interests. The first lecture by Dr. Hoare, Assistant Director of the Tin Research Institute, gave us the latest information about the methods in use at this well-known Institute. The lecture by Mr. Nickolls on 'Recent advances in forensic laboratory techniques' naturally attracted a large audience who were no doubt interested in both the scientific problems and the human elements involved in such a subject. Dr. Campbell in his talk on 'The electron microscope in studies of protein synthesis' explained the most interesting technique of which most of us have little knowledge. Dr. Lee and Dr. Gross in their respective lectures 'Radio-frequency methods in analytical chemistry' and 'High voltage paper electrophoresis' gave a most competent survey of these two subjects and Dr. Busvine in his lecture on 'The biological assay of insecticides' reviewed the difficulties of a most important problem from the point of view of world health and food supplies. Dr. Wootton's lecture on 'Automation in the medical laboratory' could be regarded as a logical sequel to the lecture on the more general aspects of the subject last year by Professor King, and Mr. Suffolk in his talk on 'Laboratory workshops' gave an entertaining review of a subject which is of the greatest importance but on which little has been written.

Dr. Stephen enlightened most of us on 'The Weisz ring-oven technique in micro-chemistry', a technique of which very few people are still aware. He made it quite clear that this had considerable scope in many laboratories. Dr. Scott gave us very up-to-date information on one of the most important of contemporary techniques in his lecture on 'Vapour phase chromatography'.

Finally, Dr. Mossel talking on 'Modern methods for the microbiological examination of foods' and Professor Frazer on 'The examination of food additives' gave masterly and highly compressed accounts of the present position on two very important subjects.

As previously it is hoped to publish all these lectures in subsequent issues of 'Laboratory Practice' and all readers will have the opportunity of studying and digesting at their leisure the enormous amount of up-to-date information which these lecturers succeeded in condensing into a small amount of time.

In response to demand from the laboratory supply industry it has been arranged to organize a similar exhibition at Harrogate in May, 1962. It is also hoped to arrange a similar series of lectures in recent advances in laboratory techniques.

Royal Society and Nuffield Foundation Awards

Among the awards under the Royal Society and Nuffield Foundation Commonwealth Bursaries Scheme are the following:

Mr. L. G. Campbell, of the Imperial College of Tropical Agriculture, Trinidad, to enable him to study aspects of drainage and irrigation in Ontario and Alberta during July and August 1961.

Dr. Rosalie F. Ewer, research associate, Rhodes University, to assist her to study fossil material of the early archosaur reptile *Euparkeria* at University College, London, for four months from September 1961.

Dr. K. A. Kermack, lecturer in zoology, University College, London, to enable him to examine in museums in South Africa specimens of reptiles which were the ancestors of the Mammalia and to make collections from the beds of Uppermost Triassic age in South Africa, from October 1961 for five months.

Dr. D. F. McMichael, curator of molluscs, the Australian Museum, Sydney, to assist him to spend about four months from October 1961 at the British Museum (Natural History) studying the collections of mollusca with special reference to the type specimens of Australian and New Guinea land and freshwater mollusca housed there.

Dr. W. Macnae, lecturer in zoology, University of the Witwatersrand, to enable him to spend six to eight months from December 1961 based on the University of Queensland and studying the ecology of the *Cymodocea* fields and mangrove swamps on shores within the Great Barrier Reef and in northern Australia.

LABORATORY EQUIPMENT TEST REPORT—No. 43

THE TINSLEY PEN-RECORDING POLAROGRAPH MARK 19

Manufacturer: Evershed & Vignoles Ltd., Acton Lane, Chiswick, London, W.4.

Price: £504 9s. 0d. (including main accessories).
Source of Supply: 200/260 or 100/120 V., A.C., 50 c.p.s.

Introduction

There is an increasing demand for instruments which enable the laboratory worker to determine, both rapidly and efficiently, the amounts of substances in solution, even when present only in traces. The Tinsley Mark 19 polarograph, which was designed to meet such requirements consists essentially of a polarizing unit, an amplifier and a pen recorder. Either direct or derivative polarograms can be produced.

Description and Mode of Operation of Instrument

A linearly increasing polarizing potential is applied across the polarographic cell by means of an auto-potentiometer, which has a clear circular scale, 6 in. in diameter and graduated in 20mV steps covering the range +0·5 to —3·0 volts. It is driven by a synchronous motor through a friction clutch so that the potential can be set, if necessary, by hand to any desired voltage. The normal auto-potentiometer speed is 1 volt in 400 secs. so that each polarogram can be traced in a few minutes. The potential is derived from a stabilized mains unit, which is standardized against a Weston cell, the balance being easily checked by depressing a button on the control panel.

The current produced from the polarographic cell is amplified from the mirror galvanometer and photocell by means of a D.C. amplifier, the output of which operates the pen arm through a moving-coil milli-

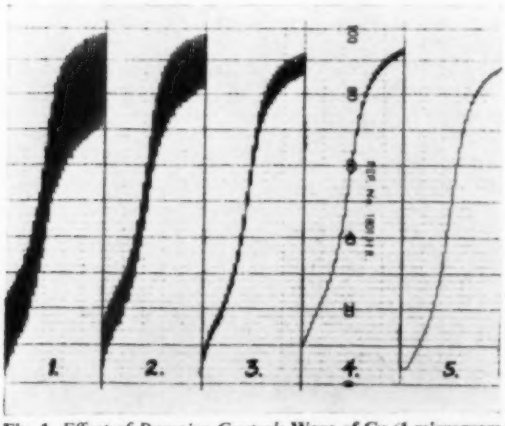

Fig. 1. *Effect of Damping Control:* **Wave of Cu (1 microgram per ml.) in 0·5M Na tartrate, pH = 5·0 (0·01% gelatin). Sensitivity 0·75 microamps. Damping No. 1 = 0; No. 2 = 1; No. 3 = 2; No. 4 = 3; No. 5 = 4 (maximum).**

The Tinsley pen-recording polarograph Mark 19.

ammeter. As the mercury drops fall into the cell the pen moves across the motor-driven roll chart which is 3½ in. wide and divided into 50 parts on the current axis (0-100 units) and in 0·1 volt on the potential axis. The standard forward auto-potentiometer speed of 0·5 volt per 100 secs. coincides with a chart movement of 1 in. at 50 c.p.s. The chart roller can also be set manually in any desired position. As the ink seems to dry up rather slowly it seems to be advisable to allow the paper to hang down from the apparatus overnight before handling in order to reduce the possibility of smudging the polarograms. Electrical coarse and fine controls (which appear to be somewhat sluggish on the instrument under test) are provided which bring the pen to zero on the chart. This can be done at any desired voltage, so that by also applying increased sensitivity, it is possible to amplify any particular small step in the polarogram.

The sensitivity knob has 12 positions covering the range 0·1-100 microamps, which represents the current for the full-scale deflection on the chart. By using this in conjuction with the range multiplier (giving factors of XI, XI·2 and XI·5—one set for the direct and another for the derivative circuit) the number of steps is

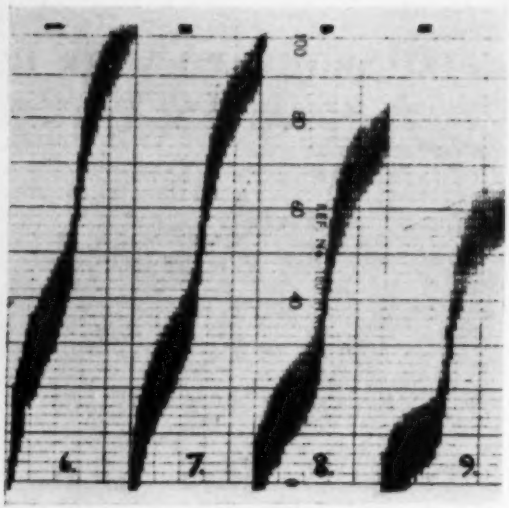

Fig. 2. *Effect of Countercurrent Control:* **Wave of Cu (0·8 microgram per ml.) in ca. 2N H_2SO_4. Sensitivity 0·5 microamps. Damping 0. Countercurrent No. 6 = 0; No. 7 = 2; No. 8 = 5; No. 9 = 8 (maximum).**

increased to 34 thus giving a total range of 0·1-150 microamps.

The counter current control, which can only be used for direct polarograms, is calibrated in 10 equal steps, the value at any setting automatically increasing as the sensitivity is increased.

A further control gives four different degrees of damping by introducing electrolytic condensers across the recorder. The manner in which this control progressively reduces the pen excursions due to the mercury drops is shown in Fig. 1.

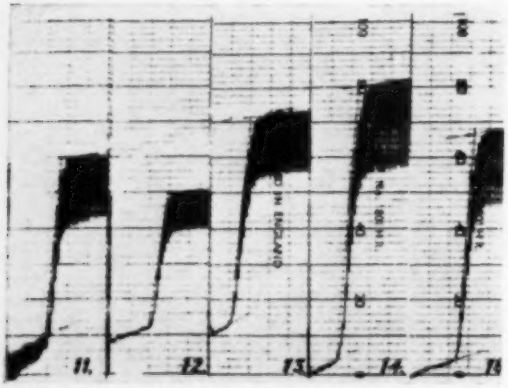

Fig. 4. *Calibration Curve: Use of Sensitivity Control:* **Waves of Cu in 0·5M Na tartrate pH = 5·0 (0·01% gelatin).**
No. 11. 10 microgram per ml. Cu, sensitivity 2 microamps;
No. 12. 20 microgram per ml. Cu, sensitivity 5 microamps;
No. 13. 30 microgram per ml. Cu, sensitivity 5 microamps;
No. 14. 40 microgram per ml. Cu, sensitivity 5 microamps;
No. 15. 50 microgram per ml. Cu, sensitivity 7·5 microamps.

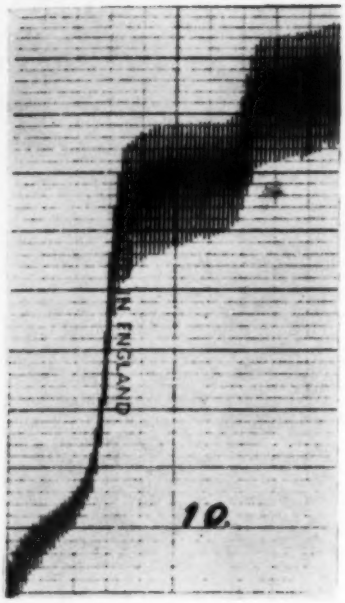

Fig. 3. *Copper plus lead:* **Wave of Cu (10 microgram per ml.) and Pb (10 microgram per ml.) in 0·5M Na tartrate, pH = 5·0 (0·01% gelatin). Sensitivity 2 microamps.**

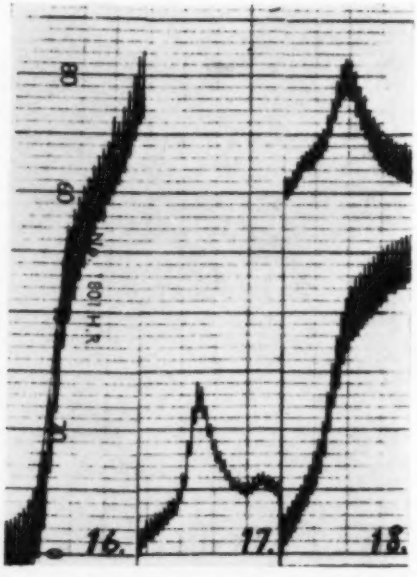

Fig. 5. *Use of Derivative Circuit:* **No. 16 = direct, No. 17 = derivative wave of Cu (1·6 micrograms per ml.) in ca. 2N H_2SO_4. No. 18 shows a typical wave of Cu in Na tartrate together with corresponding derivative wave.**

Electrode Stand

The dropping electrode system is mounted on a separate stand (39 in. high). The cell carrier can be swung round so that any one of three cells can be readily brought under the electrode tube. The latter is fitted with a 3-hole bung through which pass the anode lead, the capillary and degassing tubes. These are held in a sliding carriage, which, on loosening the fixing screw, lifts automatically due to the action of an alarmingly strong spring. The mercury reservoir, which can be locked in position on the stand at any desired height, has a polythene dust cover.

Cabinet

The main parts of the instrument are housed in an attractive polished hardwood case, which measures 18½ in. (height) × 18½ in. (width) × 14 in. (depth). The main controls are conveniently situated on the front of the instrument. The door on the right of the case, which is fitted with a sliding window, is easily removable to give ease of access to the illuminated chart, pen, etc. The use of wood for the cabinet makes it extremely durable, but in view of the considerable weight of the instrument (84 lb.), it would appear to be advantageous if the case were fitted with carrying handles.

Results

Whilst on test the Tinsley polarograph was mostly used for the determination of traces of metals (e.g. Cu, Pb, Sn) in standard solutions and in solutions obtained from the wet oxidation of foodstuffs. Lead and copper when present together showed clearly defined steps, but as one would expect with this type of polarograph, difficulties arose if the metals present had half-wave potentials which were very close to one another. For example, using N. hydrochloric acid as base electrolyte, lead and tin, which have half-wave potentials (*vs* S.C.E.) of —0·435 and —0·47 volts respectively, could not be separated even using the derivative circuit. Apart from the zero setting device, all the controls (damping, counter current, sensitivity, etc.) operated efficiently and with a positive action and it should be clear from the accompanying Figures (1-5) that the instrument produced clearly defined and reproducible polarograms.

Conclusion

The Tinsley Mark 19 polarograph can be employed for the rapid determination of extremely small concentrations of constituents such as metals. The instrument produces clear ink records of both direct and derivative polarograms and would appear to be especially useful for research purposes.

CORRESPONDENCE

To the Editor,
Laboratory Practice.

DEAR SIR, I have read with interest your editorial in Laboratory Practice, Volume 10, No. 6, on the question of large congresses. You may like to know that in this country we have attempted to eliminate the difficulties which you have correctly outlined in your editorial by arranging smaller conferences at which attendance is strictly by invitation.

For the last four years the radiation chemists have organized a conference in North Wales, known as the Miller Conference. This conference is run along the lines of the American Gordon Conference and attendance at the meeting, which is limited to not more than 100 persons, is solely by invitation. We meet to discuss only fundamental research work, and in order to promote discussion on research which is actually being carried out we do not publish any proceedings. We do not, of course, limit invitations to those who are to read a paper at the meeting but we do expect all present to join in the discussion.

A further development of this idea has been that the high polymer chemists in the United Kingdom have joined together to form the High Polymer Research Group and they organized their first meeting in Devon at Easter of this year. Again, this conference was run along lines similar to the American Gordon Conference.

We believe that in future more and more groups of scientists will organize meetings among themselves in this way and that large international conferences will only be necessary at very rare intervals. I must say that I subscribe entirely to your views on the limited value of large international conferences.

Yours faithfully,
Isotope.

New President for U.S.S.R. Academy of Sciences

Academician Mstislav Keldysh, a prominent mathematician, mechanical engineer and computer expert, has been elected as President of the U.S.S.R. Academy of Sciences. He succeeds Alexander Nesmeyanov who has resigned at his own request, pointing out that he had held this administrative office for more than ten years. Nesmeyanov himself nominated Keldysh as his successor.

Academician Keldysh graduated from the Physics and Mathematics Faculty of Moscow University in 1931 at the age of 20, and won his doctorate in 1938.

Now 50 years of age, he worked for several years in the Central Institute of Aerodynamics and originated many new trends in this field of work. Theories he elaborated have led to effective methods of eliminating 'flutter' in wings and tail units of aircraft. His theories also resulted in new designs for hydro-foil ships.

From 1932 he was first a reader, and later a professor at the Moscow State University. In 1943 he became a corresponding member of the Academy of Sciences and in 1946 was elected a full member. In 1960 he became a Vice-President of the Academy.

In mathematics Mstislav Keldysh has made major contributions to the development of calculus, to computing techniques and to various aspects of automatic remote control.

ORIGINAL CONTRIBUTION

COMPARISON OF COLONY COUNTS ON YEASTREL MILK AGAR AND ON A STANDARD REFERENCE MEDIUM

by K. Elson and S. B. Thomas

National Agricultural Advisory Service, Trawscoed, Aberystwyth

with technical assistance from

Mary C. Beavan, Mair Griffiths, H. J. Jones, Laura Jones and R. P. Middlehurst

Milk Service, Wales

YEASTREL milk agar has, since 1935, been used by dairy bacteriologists in Great Britain for the determination of the colony count of milk and also for that of rinses and swabs of farm dairy equipment. In the United States, however, the American Public Health Association (1953) recommended tryptone glucose yeast extract agar for similar work.

A compromise between the two media was introduced by the International Commission for Standardization of Methods of Analysis of Milk and Milk Products (Report 1959), which recommended that a Standard Reference Medium, the original tryptone glucose yeast extract agar plus 1 per cent skim milk, be used for this purpose.

Colony counts of milk and rinses of dairy equipment obtained with Yeastrel milk agar (YMA) prepared at different laboratories may sometimes show considerable variation, depending on the nutritive quality of the ingredients, particularly peptone and yeast extract, and on the final pH of the medium.

The object of the present investigation was to determine whether comparable colony counts could be obtained on YMA prepared at 12 different laboratories and on the Standard Reference Medium (SRM) prepared at one central laboratory. At the same time the investigation provided an opportunity of comparing colony counts on YMA prepared from individual ingredients with that made from a dehydrated complete medium of British make.

Experimental Methods

The Standard Reference Medium (SRM) was prepared at the co-ordinating laboratory at Trawscoed in batches of four litres, bottled in 100 ml. quantities in 4 oz. medical flat bottles and immediately distributed to the participating laboratories. The medium was prepared by the same person from one batch of dehydrated tryptone glucose yeast extract agar to which was added 10 ml. of skim milk per litre. Detailed composition of the medium was as follows:

Yeast extract	2·5 g.
Tryptone	5·0 g.
Glucose	1·0 g.
Agar	15·0 g.
Distilled water	1,000 ml.
Skim milk	10 ml.
pH	7·0

The Yeastrel milk agar irrespective of whether it was made from ingredients or from the dehydrated product was prepared strictly in accordance with the methods prescribed by the Ministry of Agriculture, Fisheries and Food (Report 1961). Its composition was as follows:

Yeastrel	3 g.
Peptone	5 g.
Agar	15 g.
Fresh whole milk	10 ml.
Distilled water	1,000 ml.
Final pH	7·0

Farm milk supplies were examined within 18-24 hr. of production, while rinses of farm dairy equipment were tested within 6 hr. of sampling.

Samples were examined on each occasion by means of triplicate colony counts at each of three dilutions on both YMA and the SRM, i.e. six sets of Petri plates were set up for each sample. Plates were prepared from 10^{-1}, 10^{-2} and 10^{-3} ml. dilutions of milk and from 1 ml., 10^{-1} and 10^{-2} ml. of rinse, the same tube of $\frac{1}{4}$ strength Ringer solution being used for the inoculation of the six plates at each dilution.

The order of introducing the inoculum, at each dilution level, to the plate, the order of pouring agar and the order of enumerating colonies on the countable plates was randomized. The 18 plates for any one sample were arranged in six stacks of three plates each on the same shelf of the incubator. They were incubated at 30 ± 1 °C., for 72 ± 3 hr. and the colonies then counted with the aid of a Mattick and Hiscox counting chamber, a tally counter, and a hand lens. Only those plates having a colony count between 30 and 500 colonies were recorded as countable. The arithmetic mean of the colony counts for the dilution providing the three countable plates was used for statistical analyses. The work was continued until 21 sets of countable results had become available for each of the following:

(i) Raw milk; YMA prepared from individual ingredients.

TABLE I

Arithmetic means of colony counts on triplicate countable plates incubated for 72 hr. at 30° C.

Raw Milk				*Rinses of Dairy Equipment*			
A*		B**		A*		B**	
YMA	SRM	YMA	SRM	YMA	SRM	YMA	SRM
47	31	35	60	31	40	44	39
57	76	47	47	40	31	45	39
68	91	66	56	43	39	48	47
70	65	60	57	52	42	57	53
72	77	72	60	55	36	84	79
72	68	72	60	55	56	86	107
77	75	71	70	57	54	86	106
78	60	105	102	64	61	89	77
91	117	108	103	70	65	90	95
92	62	108	116	75	65	122	125
110	117	110	115	84	114	142	142
149	141	116	113	91	139	157	126
163	147	156	149	92	90	165	174
164	171	185	181	103	114	167	171
201	193	225	220	115	125	185	152
222	227	335	272	124	145	228	269
223	229	236	303	128	123	237	259
259	250	284	276	158	161	252	320
361	356	321	354	203	176	312	319
471	455	440	381	357	319	331	370
489	473	476	422	493	428	424	436

A*—Yeastrel milk agar prepared from ingredients.

B**—Yeastrel milk agar prepared from a dehydrated commercial product (British).

NOTE: The B series was composed of entirely different samples from those included in the A series.

(ii) Raw milk; YMA prepared from dehydrated medium.

(iii) Rinses; YMA prepared from individual ingredients.

(iv) Rinses; YMA prepared from dehydrated medium.

Results

The arithmetic means of the colony counts on triplicate countable plates are given in ascending order of magnitude in Table I. It will be seen from the detailed results that appreciable differences in colony count on the two media seldom occurred. No doubt comparison can be made between the colony counts on YMA prepared from ingredients (Series A) with those prepared from a dehydrated medium (Series B), since different samples were used for the two series.

Of the 84 samples examined, 49 (58·3 per cent) gave higher counts on YMA, and 33 (39·3 per cent) gave higher counts on SRM. More milk samples gave higher counts on YMA than on SRM irrespective of whether the former had been prepared from ingredients or from the dehydrated medium. Examination of the results obtained from rinses of farm dairy equipment show however that while more samples gave higher YMA counts when it was prepared from ingredients the reverse was true with the dehydrated medium. This is the only indication which might suggest that the dehydrated medium was not quite as suitable as that made from individual ingredients.

The t test was used to determine whether the arithmetic mean of the colony counts on the two media showed any statistical significance (Table II). Values of $t = 2{\cdot}086$ and $2{\cdot}845$ correspond respectively to probabilities of 1/20 and 1/100 that the differences observed would occur by chance. It will be seen that there was no statistically significant difference between the colony counts obtained on the two types of media.

Some participants claimed that SRM was less opaque than YMA and that larger colonies, which were easier to count, developed in three days at 30° on the former, but these differences were not very marked, neither were they always evident.

Summary

A comparison of replicate colony counts on Yeastrel milk agar and a Standard Reference Medium (tryptone glucose yeast extract milk agar) showed that there was no statistically significant difference between the colony

TABLE II

Summary of comparisons of colony counts on Yeastrel milk agar (YMA) and Standard Reference Medium (SRM).

Material examined	*YMA prepared from:*	*Samples*	*Relation of YMA count to SRM count*			*t*
			Higher	*Equal*	*Lower*	
Raw milk	Ingredients	21	13	0	8	0·835
Raw milk	Dehydrated	21	15	1	5	0·839
Rinses of farm dairy equipment	Ingredients	21	13	0	8	0·627
	Dehydrated	21	8	1	12	1·457
Total samples	—	84	49	2	33	—

counts obtained on the two media, irrespective of whether the former was prepared from individual ingredients or from a dehydrated product.

The most significant result of the investigation was that comparable colony counts were obtained on YMA prepared at 12 different laboratories with that obtained on a Standard Reference Medium prepared in bulk at one laboratory and distributed to each of the participating laboratories. The YMA was prepared according to a standard technique at all the 12 laboratories and the ingredients were usually obtained from the same sources. Nevertheless, it was encouraging to find that each of the laboratories obtained comparable YMA counts with those obtained on the control SRM medium. There was no consistent difference in the relative size of the colonies appearing on the two media and they were quite as easy to count on YMA as on SRM.

This type of work provides a very useful and interesting method of control on media preparation and the determination of colony counts in any group of dairy bacteriology laboratories.

REFERENCES

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Forthcoming Conferences

BISRA Conference

BISRA Metallurgy Division are to hold a Conference ('Hydrogen in Steel') at Harrogate from October 11 to 13 next. The specific aspects of the problem to be discussed include:

1. Removal of hydrogen from liquid and solid steel.
2. The diffusion and solubility of hydrogen in steel.
3. The effect of hydrogen on the properties of ultra-high tensile steels.
4. Hydrogen in weld metal.

Full details and application forms will be available from August 14, when applications should be made to the Technical Secretary, Metallurgy Division, BISRA, 11 Park Lane, London, W.1.

Institute of Physics and the Physical Society

The Institute and Society on behalf of the International Union of Pure and Applied Physics, and the British National Committee for Physics is arranging an International Conference on The Physics of Semiconductors, which will be held at the University of Exeter from July 16 to 20, 1962. The Conference is planned to follow the previous sequence of Conferences on the physics of semiconductors, which were held in Reading in 1950, Amsterdam in 1954, Garmisch in 1956, Rochester in 1958 and Prague in 1960.

Accommodation will be provided in Halls of Residence at the University. Further information regarding the Conference may be obtained from the Administration Assistant, The Institute of Physics and The Physical Society, 47 Belgrave Square, London, S.W.1.

The British Society of Rheology

The British Society of Rheology and the Non-Destructive Testing Group of the Institute of Physics and the Physical Society will hold a joint conference on Rheological Techniques in Llandudno from Wednesday, September 20 to Friday, September 22, 1961. A wide range of techniques will be discussed, and it is hoped that a number of pieces of apparatus will be demonstrated. It is intended that the atmosphere of the meeting, which will be open to persons who are not members of the Societies, shall be informal. Workers in fields not primarily concerned with physics or mechanics will be welcome.

Details of the programme can be obtained from Dr. Peter Lord, Royal College of Advanced Technology, Salford 5., through whom bookings for accommodation can be made.

Most of the papers will be published in a special rheology number of *Laboratory Practice* towards the end of the year.

Association of Public Analysts

At the recent Annual General Meeting of the Association of Public Analysts, the following Officers were re-elected for the coming year:

President:	J. H. Hamence, M.Sc., Ph.D., F.R.I.C.
Vice President:	D. D. Moir, M.Sc., F.R.I.C.
Hon. Treasurer:	R. C. Spalding, M.A., F.R.I.C.
Hon. Secretary:	F. A. Lyne, B.Sc., F.R.I.C.
Hon. Editor:	E. C. Wood, Ph.D., A.R.C.S., F.R.I.C.

PRACTICAL METHODS FOR THE MICROBIOLOGICAL ASSAY OF THE VITAMIN B-COMPLEX AND AMINO ACIDS

by E. C. Barton-Wright, D.Sc., F.R.I.C., M.I.Biol.

PART I

Introduction

It is now 22 years since Snell and Strong (1939) published their communication on the assay of riboflavin using the lactic organism *Lactobacillus helveticus* (*casei* ξ). Although micro-organisms had been used prior to 1939 in a rather desultory way for the assay of vitamins, notably thiamine (Vitamin B_1), Snell and Strong's communication was the first to show that micro-organisms, especially the lactic bacteria, could be used with confidence for the routine assay of many members of the Vitamin B-complex in the laboratory. Later, it was discovered that certain of these lactic bacteria could also be used for the assay of some 18 amino acids, including all the so-called 'essential' acids.

As microbiological assay methods may be said now to have come of age, it was thought that a review of the various generally accepted methods of carrying out these assays would be well worth while and possibly be of some help and guidance to newcomers in this fascinating field of microbiology.

This series of articles is concerned entirely with the practical details of carrying out microbiological assays of the various members of the Vitamin B-complex and amino acids. All the methods described below have been personally tested by the author and only those which have proved to be successful in his hands have been included. In certain instances alternative methods have been described, but the method described first is always the preferred one.

The most widely used organisms for the microbiological assay of the Vitamin B-complex are certain species of lactic bacteria, but a number of X-ray mutants of the mould *Neurospora* have also been used, as well as certain strains of the bottom yeast *Saccharomyces carlsbergensis* and the chrysomonad *Ochromonas malhamensis*. Lactic bacteria are usually employed for

TABLE I

Vitamin	*Test Organism*	*Assay Range*	*Measurement of Response*
Riboflavin	*Streptococcus zymogenes**	0·005-0·04 μg.	Nephelometric Acidimetric (0·05N.NaOH)
	Lactobacillus helveticus	0·05-0·25 μg.	Nephelometric Acidimetric (0·1N.NaOH)
Nicotinic Acid (Niacin)	*Lactobacillus plantarum***	0·025-0·20 μg.	Nephelometric Acidimetric (0·1N.NaOH)
Pantothenic Acid	*L. plantarum*	0·01-0·10 μg.	Nephelometric Acidimetric (0·1N.NaOH)
Biotin	*L. plantarum*	0·05-0·5 mμg.	Nephelometric Acidimetric (0·1N.NaOH)
Thiamine	*Lactobacillus viridescens* S38A	0·0025-0·03 μg.	Nephelometric
	Lactobacillus fermenti 36	0·005-0·04 μg.	Nephelometric
Folic Acid	*Streptococcus faecalis* R	0·001-0·008 μg.	Nephelometric
Folinic Acid	*Leuconostoc citrovorum* 8081	0·001-0·008 μg.	Nephelometric Acidimetric (0·1N.NaOH)
p-Amino-benzoic Acid	*Neurospora crassa* (1633)	0·0025-0·20 μg.	'Disc-inoculum' method
Choline	*N. crassa* (34486)	0·5-4·0 μg.	'Disc-inoculum' method
Pyridoxine Pyridoxal Pyridoxamine	*Saccharomyces carlsbergensis* 4228	0·0025-0·02 μg.	Nephelometric
Cyanocobalamin	*Ochromonas malhamensis*	0·05-0·8 mμg.	Nephelometric
Inositol	(See section on cup-plate assays)		

*Formerly known as *Leuconostoc mesenteroides* (A.T.C.C. 10,100)
**Formerly known as *Lactobacillus arabinosus* 17/5

TABLE II

Since lactic organisms are more usually used for the assay of amino acids, measurement of response may be made either nephelometrically or acimetrically.

Amino Acid	*Test Organism*	*Assay Range*
DL-, D- or L- Alanine	*Leuconostoc citrovorum* 8081	5·0-25·0 μg.
L-Arginine	*Leuconostoc mesenteroides* P 60	10·0-60·0 μg.
	Streptococcus faecalis R	10·0-60·0 μg.
L-Aspartic Acid	*Leuconostoc mesenteroides* P 60	10·0-50·0 μg.
L-Cystine	*L. mesenteroides* P 60	5·0-25·0 μg.
L-Glutamic Acid	*Lactobacillus plantarum*	30·0-120 μg.
Glycine	*Leuconostoc mesenteroides* P 60	10·0-50·0 μg.
L-Histidine	*Leuconostoc mesenteroides* P 60	5·0-25·0 μg.
	Streptococcus faecalis R	10·0-60·0 μg.
L-*Iso*leucine	*Leuconostoc mesenteroides* P 60	10·0-60·0 μg.
	Lactobacillus plantarum	
L-Leucine	*Leuconostoc mesenteroides* P 60	10·0-60·0 μg.
	Lactobacillus plantarum	
L-Lysine	*Leuconostoc mesenteroides* P 60	25·0-250 μg.
	Streptococcus faecalis R	10·0-60·0 μg.
L-Methionine	*Leuconostoc mesenteroides* P 60	10·0-50·0 μg.
DL-Methionine	*Lactobacillus fermenti* 36	10·0-100 μg.
L-Phenylalanine	*Leuconostoc mesenteroides* P 60	10·0-50·0 μg.
L-Proline	*L. mesenteroides* P 60	10·0-50·0 μg.
L-Serine	*L. mesenteroides* P 60	10·0-50·0 μg.
L-Threonine	*Streptococcus faecalis* R	10·0-60·0 μg.
L-Tyrosine	*Leuconostoc mesenteroides* P 60	10·0-50·0 μg.
L-Tryptophan	*Lactobacillus plantarum*	1·0-10·0 μg.
L-Valine	*Leuconostoc mesenteroides*	10·0-60·0 μg.
	Lactobacillus plantarum	

the assay of amino acids; the X-ray mutants of *Neurospora* have not proved to be successful for this purpose.

Measurement of response will necessarily depend upon the type of organism used. With the lactic bacteria either titration of the lactic acid formed during fermentation may be used or growth estimated nephelometrically. The so-called 'cup-plate' method of assay introduced by Bacharach and Cuthbertson (1948) may also be employed in certain cases. In assays with the X-ray mutants of *Neurospora*, the 'disc-inoculum' method described by Barton-Wright and Butler (1955) may be used. In the case of the yeast *Saccharomyces carlsbergensis* response may be measured nephelometrically, or again, as with the lactic bacteria, the cup-plate method may be employed if the sample be of sufficiently high potency. With the chrysomonad *Ochromonas malhamensis* which is used for the assay of cyanocobalamin (Vitamin B_{12}), response can only be estimated nephelometrically.

A list is given in Table I of the various members of the Vitamin B-complex that can be assayed microbiologically, together with the appropriate organism to be used, the dose-range of the vitamin and the method of determining response. Table II gives similar data for amino acids. In those instances in which the names of two different organisms are given for any particular assay, the organism quoted first is the one preferred by the author.

Care and Maintenance of Stock Cultures of Assay Organisms

The response shown by the lactic organisms employed in assay work decreases with time if the customary agar stock medium (glucose, yeast-water extract agar) be used, even if the organisms are sub-cultured at weekly intervals. Various enriched media containing liver extract and tryptone have been suggested to overcome this difficulty. These enriched media have given good results in the author's hands, but the medium described below has been found to be even more satisfactory and moreover, has the great advantage that it may be used for the culture of *all* the lactic organisms normally used for assay work. The addition of thiamine (10·0 μg./ml.) and folic acid (0·1 μg./ml.) to this medium is to prevent irregularities in the behaviour of *Lactobacillus fermenti* 36 (assay of thiamine) and *Streptococcus faecalis* R (assay of folic acid).

Stock Culture Medium for Lactic Assay Organisms

Glucose	4·0 g.
Peptone (Difco)	2·0 g.
Tryptone (Oxoid)	2·0 g.
Yeast Extract (Difco)	0·8 g.

Proteolysed liver	0·8 g.
Sodium acetate (hydrated)	6·4 g.
Sodium chloride	2·0 g.
KH_2PO_4	0·8 g.
Thiamine	4,000 µg.
Folic acid	40·0 µg.
Inorganic salt solution (see below) ...	2·0 ml.
Water to	400 ml.

Salt solution

	g.
$MgSO_4 . 7H_2O$	10·0
$FeCl_3 . 6H_2O$	0·1
$MnSO_4 . 4H_2O$	0·5
Water to	250 ml.

(5 drops conc. HCl added to prevent precipitation)

The pH of the medium is adjusted to 6·8 with sodium hydroxide solution and it is divided into two 200 ml. portions.

A. *Stock Medium Broth*. 10 ml. aliquots of medium are transferred to 1 oz. screw-capped bottles and sterilized at 10 pounds pressure for 10 minutes.

B. *Stock Medium Agar*. 3·0 g. of agar are added to the second 200 ml. portion of medium. The medium is steamed to melt the agar and 10 ml. aliquots are transferred to 1 oz. screw-capped bottles and sterilized at 10 pounds pressure for 10 minutes.

Broth and stab media are stored at room temperature and will retain their activity for one month. They should be discarded after this time.

The following procedure is advised for the sub-culturing of lactic organisms: A portion of the agar stab culture is transferred by means of a sterile needle to a bottle of broth and incubated for 18-20 hours at the temperature appropriate for the organism. There should be heavy growth at the end of this period. A stab culture is then made from the broth in the usual way. After inoculation the stab culture is incubated for 18-20 hours at the requisite temperature for the organism and then held in a refrigerator. All lactic organisms should be *sub-cultured at weekly intervals* if they are to be maintained in a suitable condition for successful assays.

Incubation Temperatures for Lactic Organisms

Lactobacillus fermenti 36	37°C.
L. helveticus	37°C.
L. plantarum	30°C.
L. viridescens	30°C.
Leuconostoc citrovorum 8081	37°C.
L. mesenteroides P 60	30°C.
Streptococcus faecalis R	30°C.
Str. zymogenes	37°C.

Neurospora Mutants

It was formerly recommended that stock cultures of *Neurospora* mutants should be carried on peptone, yeast, malt agar slopes. The disadvantage of this medium is that there is excessive vegetative growth and little sporulation. With the potato agar medium described below mycelial growth is reduced and the moulds spore well.

Preparation of Potato Agar Slopes.—The tubers are well washed, peeled, cut into small portions, and 100 g. weighed into a beaker, autoclaved at 15 pounds pressure for 15 minutes and thoroughly mashed into a fine paste with a fork. Next 8·0 g. of agar dissolved in 400 ml. of distilled water are added slowly with constant stirring to the mashed potatoes, the medium is tubed, the tubes plugged with cotton wool and sterilized at 15 pounds pressure for 15 minutes.

Neurospora mutants should be sub-cultured at fort-nightly intervals. After inoculation the tubes are left at room temperature in the light to encourage sporulation.

Saccharomyces carlsbergensis 4228

Cultures of *Saccharomyces carlsbergensis* 4228 are carried on 4 per cent malt agar slopes at a pH of 5·0 to 5·5. Sub-culturing is carried out at weekly intervals by transferring a portion of the yeast growth by means of a sterile needle to a tube of sterile 5 per cent malt extract, incubating for 18-20 hours at 25-28°C. and

TABLE III

Vitamin	*Test Organism*	*Known Interfering Substances*
p-Aminobenzoic Acid	*Neurospora crassa* (mutant 1633)	
Biotin	*L. plantarum*	Fatty Acids
Choline	*Neurospora crassa* (mutant 34486)	Methionine
Cyanocobalamin	*Ochromonas malhamensis*	
Folic Acid	*Streptococcus faecalis* R	Thymine
Folinic Acid	*Leuconostoc citrovorum* 8081	
Nicotinic Acid	*Lactobacillus plantarum*	Fatty Acids
Pantothenic Acid	*L. plantarum*	Fatty Acids
Riboflavin	*Streptococcus zymogenes*	
Thiamine	*Lactobacillus viridescens* S38A	
Vitamin B_6-Complex	*Saccharomyces carlsbergensis* 4228	

than making a slope from the liquid culture in the usual way.

Ochromonas malhamensis

Stock cultures of *Ochromonas malhamensis* are maintained on the following liquid medium:

Glucose	0·2 g.
Tryptone (Oxoid)	0·1 g.
Proteolysed liver (Paines and Byrne)	0·2 g.
Distilled water	200 ml.

Ten ml. aliquots of the medium are transferred to 50 ml. conical flasks, which are plugged with cotton wool and sterilized at 15 pounds pressure for 15 minutes.

Sub-culturing is carried out twice a week. It is advisable to have at least two series of stock cultures, or better still three, running simultaneously in case one of the flasks becomes contaminated. One ml. of a previous stock culture is transferred under strictly aseptic conditions to a fresh flask of medium and the cultures are maintained at 25-28°C. under a 25 watt bulb.

Measurement of Response

As far as the lactic bacteria are concerned, response may be measured either acidimetrically by titration with 0·1 N, or in some cases 0·05 N NaOH, to pH 6·8 using bromothymol blue as indicator, or nephelometrically using any suitable type of instrument. The author has now abandoned acidimetric determinations for his own work, not only because of the saving of time (24 hours instead of 72 hours incubation), but the measurements themselves are less tedious and more expeditiously carried out in a nephelometer. The author uses an EEL portable colorimeter which is a particularly suitable instrument for this type of work. With the EEL colorimeter, the tubes containing the cultures are placed directly in the instrument and there is no waste of time transferring each culture to a special cell. The tubes used for assays are calibrated once and for all to ± 0·1 on the instrument scale with distilled water and never used for any other purpose.

Recommended Methods for the Microbiological Assay of Vitamins

The author's own personal preference of organisms which should be used for the microbiological assay of vitamins is given in Table III, together with a list of known interfering substances.

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Barton-Wright, E. C. and Butler, N. J. (1955). Analyst, **80**, 4.
Snell, E. E. and Strong, F. M. (1939). Ind. Eng. Chem. Anal. Ed., **11**, 346.

(To be continued)

Paul Instrument Fund Committee

Awards of grants by the Paul Instrument Fund Committee have been made up as follows:

- £1,550 to Dr. E. T. Hall, director of the Research Laboratory for Archaeology and the History of Art, Oxford, for the design and manufacture of a recording instrument combining a drum recorder and servo transmitters with a spectrometer to give direct reading of a wavelength.
- £3,900 to Dr. P. B. Hirsch, lecturer in physics, University of Cambridge, for the construction of an electron optical instrument which will measure the energy distribution of the electron beam passing through small areas (diameter less than 1μ) in the crystal specimen which can be observed and selected on the electron microscope image.
- £3,000, in supplement of a previous grant, to Dr. H. Motz, Donald Pollack Reader in engineering sciences, University of Oxford for the construction of a linear accelerator working at 1·6 cm. (J-band).
- £2,284 to Mr. B. M. Turner, who is working in the department of zoology and comparative anatomy, Oxford, for the further development of microscale chromatography and electrophoresis techniques.

Plastics Research

To meet the rapidly increasing technological demand of the nation's plastic industry, the former Research Association of British Rubber Manufacturers has extended the scope of its activities during 1961 under the new title of the 'Rubber and Plastics Research Association of Great Britain' in order to cover the requirements of both the rubber and the plastics industries.

The new joint research association is a logical move as the science and technology of these industries have much in common. The idea has since been widely accepted by the two industries and has received active support from members of the former Research Association of British Rubber Manufacturers and of the British Plastics Federation. The Department of Scientific and Industrial Research has also welcomed this move and has agreed to grant the new research association up to £77,000, provided industry itself raises £120,000 in each of the three years 1961-63.

At a recent technical meeting in London there was a valuable exchange of views between officials of the research association and representatives of plastics firms. Various proposals were made for future work programmes. These included standardization of test methods; productivity studies, particularly in moulding and fabrication; liaison with other research associations and laboratories on such common problems as toxicity; and a study of plastics rheology, i.e. the science of flow of matter, and the critical study of elasticity, viscosity, and plasticity.

The rapidly growing plastics industry consists of hundreds of firms embracing some relatively few producers of the raw material using science based manufacturing methods, and many small and medium-sized firms who mould and fabricate plastics articles.

New laboratories to house plastics research are now being built at the research association's headquarters at Shawbury near Shrewsbury, and will be ready for occupation within 18 months. Much of the work at first will be concerned with practical problems of process productivity and technological development. For example, there is no suggestion of entering the field of polymerization. Again on the practical level, it is probable that the research association will buy the more expensive testing machines on which measurements can be made on behalf of individual member firms. Confidential sponsored work, analyses, and the pooling of information are other ways in which the research association should be able to provide useful services of co-ordination; and a suggestion for a consumer advice service, which would assess materials on a standard basis on behalf of member firms, has received wide support.

THE FUNDAMENTAL ASPECTS OF GAS CHROMATOGRAPHY

by M. A. Khan, B.Sc.(Pb.), M.Sc.(Alig.), Ph.D.(Cantab.)

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PART I—BASIC PRINCIPLES

Introduction

Reed[1], in 1893, was the first to describe the separation of mixtures on powdered materials. No progress was made on these lines until 1906, when Tswett[2], a Russian botanist, applied the procedure to the separation of plant pigments. Tswett ran a petroleum extract of dry leaves through a vertical glass tube filled with finely divided calcium carbonate. He found that the different constituents of the extract appeared on the surface of the powder as a series of coloured bands. This series of coloured bands was called by Tswett the *chromatogram* and this particular process of separation was named *chromatography**.

Tswett's technique is simple. After the partial separation of the components of the solution (extract) into various bands, pure solvent is run into the column continuously. The various bands begin to travel down the column at different speeds. Provided the column is of sufficient length, the overlapping bands will distinctly separate from each other after percolation of large quantities of the pure solvent.

Although Tswett continued his interest in the field up to 1914 and published about 20 papers, no work on his lines was carried out for many years. However, in 1931, Kuhn and co-workers[3] applied the technique to resolve plant carotene and xanthophylls into their components. Since then the interest has not only been kept alive but expanded remarkably.

Tswett's adsorption technique, or the so-called conventional chromatography, has undergone considerable evolution and its scope has been usefully extended by new techniques discovered independently by (*a*) Swedish and (*b*) British investigators.

(*a*) During the years 1940-1942, Tiselius and Claesson[4,5] at Uppsala introduced the use of 'Frontal Analysis' and 'Displacement Development'. These techniques differ from the conventional Tswett adsorption technique in the nature of developers used for washing the chromatogram down the column. In frontal analysis the developer is the solution itself rather than the pure solvent, whereas in displacement development the developer is a fluid which is more strongly sorbed by the stationary phase than any of the constituents of the chromatogram.

(*b*) In 1941, Martin and Synge[6] introduced partition chromatography. It is based on the difference between the partition coefficients of the components between a stationary phase held on an inert solid and a flowing solvent immiscible with the stationary phase. The discovery of the partition technique has greatly extended the field in which chromatography can be usefully employed. When the moving solvent or the moving phase is a gas, the technique is called gas chromatography. The most important advantage gained by replacing the moving liquid phase by the moving gas phase lies in the fact that equilibria involving gases are set up more rapidly. This makes the chromatographic column work more efficiently and speedily than the corresponding column in which the moving phase is a liquid.

The techniques mentioned in (*a*) and (*b*) eliminate the strong 'tailing' of bands which usually occurs in conventional adsorption chromatography. At the same time, the application of chromatography has been usefully extended to colourless substances. Colour is not the only physical property which can be used to characterize the various bands. In principle, it should be possible to utilize other physical properties of the constituents to differentiate them. With the advent of modern sensing devices or detectors, it is possible to characterize the different bands of a chromatogram in a manner that is more efficient and reliable than the empirical ones used in classical chromatography. Nevertheless, the terms 'chromatogram' and 'chromatography' are still retained, being time-honoured, traditional and convenient.

In passing it may be remarked that the strongly polar compounds having, for example, carboxylic or hydroxyl groups may alter their chemical structure on solid adsorbents. The use of adsorption chromatography cannot, therefore, be recommended in such cases. Partition chromatography does not suffer from this drawback. Moreover, substances in the adsorbed form may be sensitive to oxygen and light, but partition chromatography is free from such limitations.

Theoretical

From the practical view point, a study of the theory of chromatography is of immense value. It gives insight into the different mechanisms influencing the band width on which, primarily, depends the entire process of separation. The factors which affect the band width and hence the column efficiency are: (i) sample size, (ii) amount of stationary phase, (iii) solute—stationary phase interactions, (iv) size of the particle holding the stationary phase, (v) flow rate and velocity distribution of the flowing phase, (vi) column length, (vii) column diameter, (viii) column temperature, and (ix) the uniformity of packing.

*Χρωματιον = khromation = colour, Γραμμα = gramma = picture, γραφειν = graphein = to write. Thus chromatogram literally means a 'coloured picture' and chromatography 'writing in colour'.

It is highly desirable to understand the basic principles associated with these factors, and find a correlation, between them and the band width, which serves as a guide for the choice of best conditions for resolving mixtures.

The first step in this direction was taken by Wilson[7]. He calculated the behaviour of a single solute on a chromatographic column. A fairly general theory of the chromatographic process was later advanced by de Vault[8]. This is based on the assumptions:

(A) There is no diffusion in the moving phase along the axis of the column.

(B) There is instantaneous equilibrium at all points of the column. This means that the rate constants for the sorption and desorption processes are infinitely large and thus the time taken for the equilibrium to be established, between the concentrations of the solute in the stationary and mobile phases, can be neglected.

The validity of these two assumptions will be examined simply and critically in Part II of this series.

To understand the fundamental nature of the chromatographic process, without undue complications, it is advisable to study the chromatographic behaviour of solutions containing one solute only. Logically, once this simple problem is solved, the more complex problem of the chromatography of mixtures can then be tackled relatively easily. The simplified treatment, given below, is essentially due to Weiss[9] and de Vault. A few slight modifications, however, have been introduced in order to make the theory applicable to both adsorption and partition columns.

Simplified Theory of a Single-Solute Chromatogram

Let A = area of cross-section of the chromatographic tube, c = concentration in the mobile phase, i.e. no. of g. of solute in one ml. of mobile phase, C = concentration in the stationary phase, i.e. no. of g. of solute in one g. of stationary phase.

In general, C will be some function of c which may be represented by $C = f(c)$.

For linear distribution isotherms*: C = ac so that

$$\frac{C}{c} = a = \frac{dC}{dc} = \frac{df(c)}{dc} = f'(c).$$

With these definitions of C and c, we note that a will not be a dimensionless number.

If K is the dimensionless partition coefficient, as commonly defined in partition chromatography then: $a = K/\rho$ where ρ is the density of the stationary liquid phase held on the solid.

L = length of the column (cm.)

x = distance measured from the column top (cm.)

M = weight of the stationary phase (g.)

$m = \frac{M}{L}$ = wt of stationary phase per unit column length (g. cm^{-1})

v_0 = initial volume of the sample of fixed concentration c_0 introduced into the column ($cm.^3$)

x_0 = length (or width) of the initial band formed after the complete entry of v_0 ml. of the sample (cm.)

α = free volume or pore space between the particles per unit column length ($cm.^2$)

v = volume of the flowing solvent (mobile phase) passed through the column after the formation of the initial band.

V_R = retention volume ($cm.^3$)
volume of the mobile phase passed through the column until the band front reaches the column outlet.

Now consider a thin layer of the band between x and $(x + \delta x)$ after a volume v of the mobile phase has been added to develop it. Watch the passage through this layer of an element of volume δv of the mobile phase at concentration c. The passage of δv will bring about a change both in the concentrations of the mobile and the stationary phases contained within the layer. The law of conservation of mass, according to which the amount of solute which goes into the layer must be equal to the amount which comes out plus what is retained within the layer, leads to:

$$\frac{\partial c}{\partial x} + \left[\alpha + m\,f'(c)\right]\frac{\partial c}{\partial v} = 0 \qquad \ldots\ldots\ldots\ldots (1)$$

The solution of this partial differential equation is given as:

$$c = \Phi\left(v - [\alpha + m f'(c)]x\right) \qquad \ldots\ldots\ldots\ldots (2)^*$$

The solution given in (2) is perfectly general and governs both the formation of the initial band and also its development down the column.

Equation (2) describes the distribution of solute within the column with the volume of the mobile phase passed through the column. Provided the properties of the arbitrary function Φ and those of the distribution isotherms governing the behaviour of $f'(c)$ are known, it is possible to derive a picture of the chromatographic process for a single solute under the conditions of instantaneous equilibrium and no diffusion along the column in the mobile phase. Mathematical analysis of equation (2) reveals the following facts.

(1) For a sample of volume v_0 and concentration c_0 the initial band width** (x_0) is given by:

$$x_0 = \frac{v_0}{\alpha + m\,\dfrac{f(c_0)}{c_0}} \qquad \ldots\ldots\ldots\ldots (3)$$

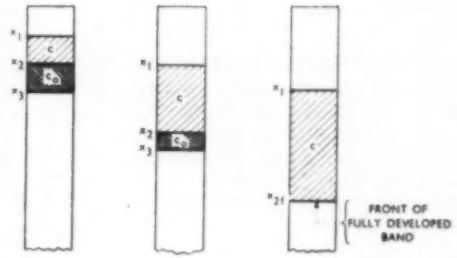
*A distribution isotherm refers to the plot of concentration in the stationary phase versus concentration in the moving phase. For a gas-liquid partition column, a linear isotherm corresponds to Henry's law which simply states that the amount of vapour absorbed in the liquid phase is proportional to the first power of its concentration in the vapour phase.

*The original equation (1) can be easily obtained by eliminating the arbitrary function Φ by partial differentiation.

**Band width refers to the distance between the rear and front boundaries of the band.

FORMATION OF INITIAL BAND

FIG. 1



SUCCESSIVE STAGES OF DEVELOPMENT OF A SINGLE SOLUTE BAND

FIG. 2

The front of the band is sharp*, and its concentration over the entire width is uniform (Fig. 1).

(2) On the addition of mobile phase, the initial band begins to move down and its structure** no longer remains constant and homogeneous. As soon as the mobile phase enters the column, it begins to strip off solute from the stationary phase right from the top end. This process of stripping off continues until the front portions of the advancing mobile phase have reached the concentration c_0. The front of the moving band remains sharp and migrates with a velocity which would be the same as would be expected if solution at concentration c_0 were continuously admitted into the column. The band can be divided into two regions of different concentrations. The distances (x_1, x_2, x_3) along the column length (Fig. 2), theoretically covered by the band during the process of development, are calculated as†:

$$x_1 = \frac{v}{\alpha + m'f(o)}$$

$$x_2 = \frac{v}{\alpha + m'f(c_0)}$$

$$x_3 = x_0 + \frac{v}{\alpha + m\dfrac{f(c_0)}{c_0}} \qquad (4)$$

where $'f(o)$ and $'f(c_0)$ are the first derivatives of $f(c)$ evaluated at $c = 0$ and $c = c_0$ respectively, and x_3 represents the distance travelled by the band front at constant concentration c_0.

*This is derived from the discontinuous nature of function ϕ.
**The band structure refers to both concentration and width of the band.

†The upper part (x_1x_2) of the band will always move with a variable concentration c which is a function of $\frac{x}{v}$. The lower part (x_2x_3), as pointed out already, continues to move with the original concentration c_0 until the band is just completely 'washed out' or just fully developed.

The upper part of the band x_1x_2, where the concentration c gradually varies as the band is washed down the column, is known as the variable part of the band. It is also called the rear of the band or the 'tail' of the band. As we shall see presently, regions of variable concentrations do not occur with linear sorption isotherm, because the band on development moves down with a constant width over which the concentration is always constant and uniform. It is obvious from Fig. 2 that when $x_2 = x_3$, the lower part x_2x_3, of the band at concentration c_0 just disappears. When this happens, the band is fully developed. The minimum volume, v_m, of the mobile phase required to bring about this change is obtained by equating the expressions for x_2 and x_3 given in (4). Thus

$$v_m = v_0c_0 \cdot \frac{\dfrac{\alpha}{m} + 'f(c_0)}{f(c_0) - c_0'f(c_0)} \qquad \ldots\ldots\ldots\ldots (5)$$

Shape of the Chromatographic Band

When equilibrium between the two phases is instantaneous and there is no longitudinal diffusion,† the shape of the band depends upon the distribution isotherm.

(*i*) *Linear Isotherms*

We have already pointed out on page 548 that for linear isotherms:

$$\frac{f(c_0)}{c_0} = a,\ 'f(c) = a \text{ and } 'f(c_0) = 'f(o) = a$$

On making these substitutions in equations (3) and (4) we obtain:

$$x_0 = \frac{v_0}{\alpha + ma} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (6)$$

and the width of the band when the band front has reached the point x_3 is:

$$x_3 - x_1 = \frac{v_0}{\alpha + ma} = x_0 \quad \ldots\ldots\ldots\ldots\ldots\ldots (7)$$

Equation (6) shows that for linear isotherms, the width of the initial band depends upon the sample volume and is independent of the initial concentration c_0. Equation (7) expresses the fact that the band moves down the column with a constant width i.e. the band does not broaden on its way down the column.

In practical chromatography the relative rate of migration of the band, defined as:

$$R_F = \frac{\text{velocity of the band}}{\text{velocity of the moving phase}}$$

is of fundamental importance. We note that when the mobile phase travels a distance $\frac{v}{A}$, the band front, in the same time, travels a distance of $x_3 - x_0$. Therefore, the rate of migration of the band with respect to the mobile phase for linear isotherm is given by:

$$R_F = \frac{x_3 - x_0}{\frac{v}{A}} = \frac{A}{v}(x_3 - x_0) = \frac{A}{v}\left(\left(x_0 + \frac{v}{\alpha + ma}\right) - x_0\right)$$

$$\therefore R_F = \frac{A}{\alpha + ma} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (8)$$

Provided the mobile phase travels with a uniform velocity, the above equation shows that for a given column and a given pair of the mobile and stationary phases, the band migrates down the column with constant speed.

The constancy of speed and width show that the band is of rectangular shape as shown in Fig. 3.

Putting $x_3 = L$, $v = v_R$ and $f(c_0)/c_0 = a$, in the last expression given in (4), we get:

$$L = x_0 + \frac{v_R}{\alpha + ma}$$

$$= \frac{v_0}{\alpha + ma} + \frac{v_R}{\alpha + ma}$$

$$= \frac{v_0 + v_R}{\alpha + ma}$$

$$= \frac{v_R}{\alpha + ma}$$

or

$$V_R = \alpha L + mLa = \alpha L + mLK/\rho$$

or

$$V_R = V_G + KV_L \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (9)$$

where V_R is the retention volume as defined previously, V_G is the total pore space of the column and V_L is total volume of the stationary liquid phase. This is the formula derived by Martin and Synge[5] for the partition chromatographic column. The retention volume V_R is a factor of paramount importance. It is not only used as a basis for checking the identity of substances, but also it can be used to study the thermodynamics of solutions.

(ii) Non-Linear Isotherms

For non-linear isotherms, the situation is not so simple as described for linear isotherms. Consider for example, an isotherm of the Langmuir type:

$$C = f(c) = \frac{ac}{1 + bc} \quad \ldots\ldots\ldots\ldots (10)$$

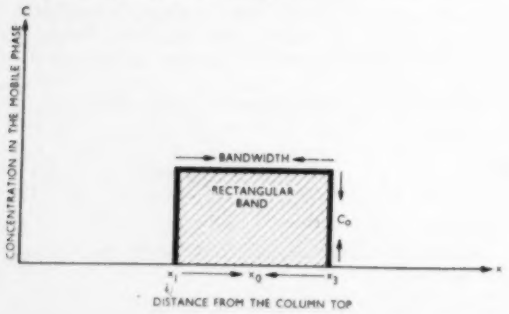


FIG. 3

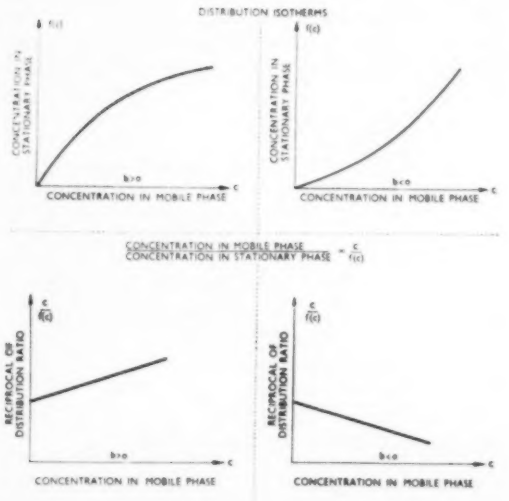


FIG. 4

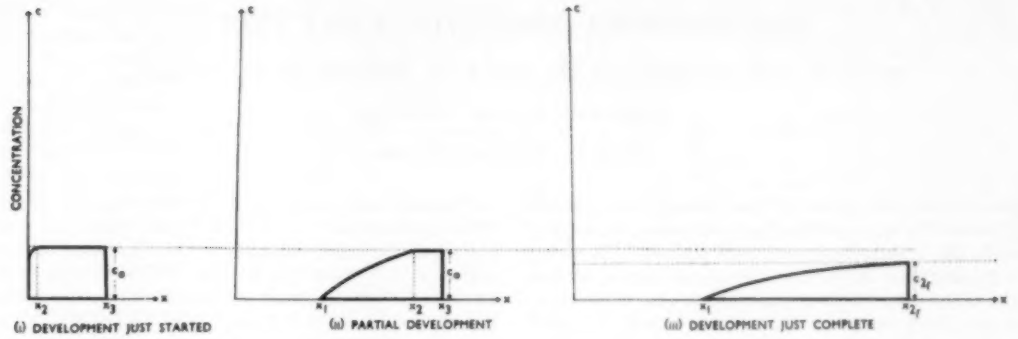


FIG. 5

where a and b are constants characteristic of the system.

When b is positive, the distribution ratio decreases with the increase in concentration. On the other hand, when b is negative the distribution ratio increases with the increase in concentration c of the solute. The two cases are shown graphically in Fig. 4.

Let us consider the shape of the band when $b > 0$. In this case we find:

$$\frac{df(c)}{dc} = f'(c) = \frac{a}{(1 + bc)^2} \quad \ldots\ldots (11)$$

Putting $c = o$ in (11) and $c = c_0$ in (10):

$$f'(o) = a \quad \ldots\ldots\ldots\ldots (11/a)$$

$$f(c_0)/c_0 = \frac{a}{(1 + bc_0)} \quad \ldots\ldots\ldots (11/b)$$

Making these substitutions in the expressions for x_1 and x_3 in (4) and then subtracting, we get:

$$x_3 - x_1 = x_0 + b \frac{mac_0v}{\{\alpha(1 + bc_0) + ma\}\{(\alpha + ma)} \quad \ldots\ldots (12)$$

Equation (12) shows that as the band is washed down the column, its width does not remain constant but is increased by an amount given by the second expression on R.H.S. of (12). For $b = o$, when the isotherm becomes linear, the band width $(x_3 - x_1)$ reduces to x_0, showing thereby that the band width remains constant.

We note that in the variable part (x_1x_2) of the band the concentration c of the solute is less than that in the constant part (x_2x_3). This means that the distribution ratio in the variable part or the tail is higher than the distribution ratio in the constant part. As a result of this, the upper part (x_1x_2) or the tail would travel more slowly than the front part. Naturally, this gives rise to spreading of the band into a tail of gradually decreasing concentration. As the development continues more and more of the solute is retained in the tail. Finally, the tail becomes so long that no portion of the band has as high a concentration as c_0. The band is now fully developed The successive stages of development are shown in Fig. 5.

In (Fig. 5-iii) the point x_1, where the concentration c is zero, represents the rear edge of the tail, whereas the point x_{2f} represents the front edge of the tail. Provided there is equilibrium between the two phases,

(*Continued on page 562*)

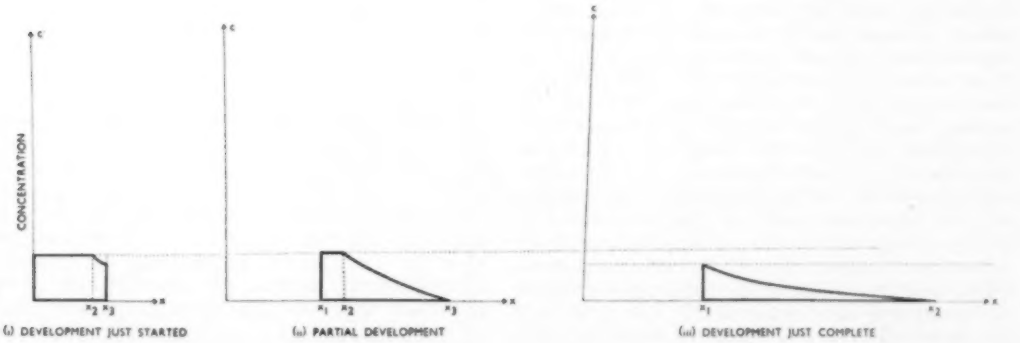


FIG. 6

THERMOGRAVIMETRIC ANALYSIS

by G. M. Lukaszewski, Ph.D., and J. P. Redfern, Ph.D., A.R.I.C.

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PART II—APPLICATIONS

IN the previous article the basic principles of thermogravimetry were outlined and some essential requirements for a thermobalance were discussed. A survey of the literature shows that thermogravimetric analysis has as yet found little application in chemistry except in the purely analytical field where French, Japanese, and Russian workers have been the most active. Nevertheless the technique is now being more widely used. Surface adsorption, phase relationships, sublimation of compounds as well as studies on mixtures have all been investigated successfully using thermogravimetric techniques. The applications may be conveniently discussed under the following headings:

(i) Applications in analytical chemistry.
(ii) Applications in physical chemistry.
(iii) Applications in inorganic chemistry.
(iv) Applications in organic chemistry.

Applications in Analytical Chemistry

The most successful application of thermogravimetric analysis to date has been the investigation of analytical procedures. Extensive work has been carried out by Duval and his collaborators since 1947. Kobayashi (1940) has published a review incorporating the results obtained principally by Japanese workers. The pyrolysis curves of a large number of inorganic gravimetric precipitates have been examined. Duval (1953) has been able to recommend some 200 out of almost 1,000 compounds examined as being suitable—in that they provide a stable weighing form within specified limits of temperature. The direct aim of a thermogravimetric investigation is to obtain information relating to the decomposition and drying characteristics of recommended gravimetric precipitates, the requirement being a horizontal portion of the curve in a plot of the weight-loss against temperature graph. In gravimetric analysis it is essential that the operator should know with certainty that the temperature of ignition or drying of a precipitate is one that will produce a material of reproducible stoichiometry. It would, for instance, be undesirable to expect precise quantitative information from a product which contained an indeterminate and variable amount of water of crystallization, or which was in the process of decomposing and consisted of a mixture of solid phases with no clearly defined relationship to the material being determined. Using this approach Beamish and McBryde (1959) have shown that certain instructions given for the heating or ignition of materials in some gravimetric determinations were quite inadequate. Often the reason for this stems from the fact that the heating temperatures for some procedures have been arrived at only empirically, and have frequently been quoted in the literature without any critical investigation of the conditions actually required in the treatment of the material concerned. Duval and his co-workers have shown that the materials employed for filtering must also be examined in this way. Although porous silica crucibles are constant in weight up to 1,000° (after an initial ignition) and sintered glass crucibles are constant up to 500°, asbestos is an extremely unreliable filtering medium because a sample may well show a steady loss in weight over the range of 300-900°. Further to this it has been shown that complete combustion of ashless filter paper is only achieved above 700°.

Fig. 8 shows a material (b) which is suitable for gravimetric estimation as it possesses two plateaux at which the composition would be known with certainty, whereas (a) decomposes continuously and hence could not be used in estimations except perhaps for ignition at very high temperatures. When a plateau is obtained it matters little whether it extends from 75-132° or from 74-131°. A temperature in the middle of the range, say 100° in the example quoted, would always yield the correct composition for the material. In the case where a new method of determination has been suggested, a thermogravimetric analysis is of the utmost value in determining whether or not the method is worthwhile, as it is immediately apparent if suitable plateaux of constant weight over a temperature range are found. Duval and also Wenger and co-workers (1945) have shown that the conditions of precipitation are extremely important. Extensive work has been carried out on the metal hydroxides from this point of

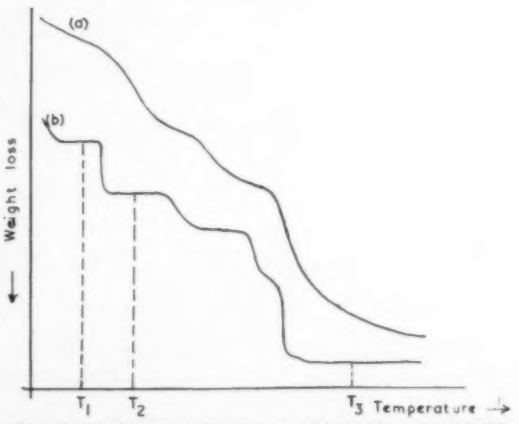

Fig. 8. Gravimetric estimation. (a) Unsuitable. (b) Suitable.

view. Here it has been shown that the nature of the precipitate, the time it is allowed to age and the speed and temperature of drying play a large part in deciding whether or not the method can be adopted for use. The factors which are important in the selection of a method for the gravimetric estimation of a component in a material can be summarized as follows:

(*a*) That precipitation is complete.

(*b*) That the precipitate is easy to handle (i.e. easy to collect, does not adhere to glass, precipitates quickly, and does not readily become contaminated by absorbing extraneous ions from solution).

(*c*) That the composition of the precipitate (dried or after ignition) is constant, i.e. that a known proportion by weight of the precipitate always corresponds to the desired component. This implies the existence of suitable horizontal portions on the pyrolysis curve.

(*d*) That the precipitating reagent is preferably neither toxic nor too expensive.

(*e*) That some variation in the conditions of precipitation can be tolerated.

A further application of the technique in the analytical field is for the assessment of the purity of materials. Thus it has been successfully applied to analytical grade reagents and especially to those materials used as primary standards in volumetric analysis, e.g. borax, sodium carbonate, ammonium oxalate, and potassium hydrogen phthalate. In the latter case the exposure of such materials to moisture causes them to absorb appreciable amounts of water. The extent of this absorption can be studied using the thermobalance and the best drying temperature can be readily found. One of the best examples of the application of the thermobalance to the investigation of a new procedure and in the assessment of purity is provided by the work of Duval on gallium. Using a Chevenard thermobalance he was able to show that in all the reagents used to precipitate gallium hydroxide, co-precipitation of iron impurities from the commercially available material resulted and the pyrolysis curves for gallium showed a rise at 900° due to the oxidation of the iron present. The one exception to this was sodium hydrogen sulphide which enabled gallium to be obtained free from iron, allowing of a considerable improvement in the values obtained for certain physical constants.

The precipitates of many hydroxides are colloidal and require considerable ageing before they become filterable. Thermogravimetric techniques are most suitable in assessing the amount of impurities present and to find the minimum temperature at which the oxides attain constant weight. Studies have shown, contrary to general opinion, that the ease with which precipitates may be filtered does not depend on the crystal form, but rather on the nature of the structure of the molecules. Also it seems that better results are obtained with weak organic bases than with either sodium or potassium hydroxides.

In the first article it was shown that plateaux, or inflections in weight-loss against temperature curves

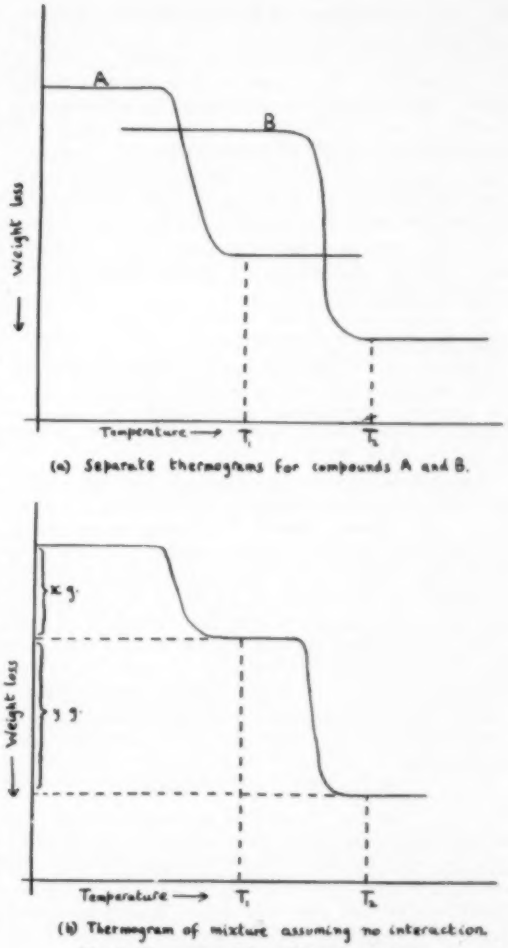

Fig. 9. The composition of binary mixtures.

could indicate the existence of compounds of definite composition, and that their resolution depended on the recording technique. Thus whereas a high rate of heating may fail to indicate the formation of stable phases of fixed composition at any given temperature, a slower rate of heating or even a quasi-static approach frequently may reveal stable phases which could be employed in gravimetric procedures and even lead to the discovery of new intermediates in the process of thermal degradation. Claisse, East and Abesque (1954) have criticized the work of Duval and his collaborators on these grounds, because many of the pyrolysis curves were obtained using high rates of heating.

A further application of thermogravimetry to analysis is in the study of the composition of binary mixtures. Thus where two compounds A and B both possess characteristic but different pyrolysis curves then the resultant curve for a mixture will be determined by

the relative proportions of A and B. This is depicted in Fig. 9a and b. If the loss up to T_1 is x g. and from T_1 to T_2 is y g. it can be seen that up to T_1 the loss is due entirely to the decomposition of A, and between T_1 and T_2 due to the decomposition of B. It is therefore possible to evaluate the relative amounts of A and B present in the mixture. This method may frequently be used to estimate the amount of co-precipitated material by a comparison of the pyrolysis curve of the suspected mixture with that of a pure sample.

It is clear that thermogravimetric analysis can supply the analytical chemist with a considerable body of valuable information.

Applications in Physical Chemistry

Thermogravimetric analysis can be applied to the study of solid-solid transformations in the following ways (*a*) kinetics of reactions, (*b*) reaction mechanisms, (*c*) phase relationships, (*d*) surface adsorption and (*e*) catalytic effects.

As yet little work in these fields has been published, probably due to the uncertainties of such matters as the effect of different shapes of crucibles on the results obtained, on the bulk of the material and how it is distributed. Vallet (1932 and later) has carried out systematic investigations of the effect of varying the rate of heating, shapes of crucibles, as well as rates of evolution and the composition of gaseous by-products for the decomposition of various materials. Prout and Tompkins (1944) studied the thermal decomposition of potassium permanganate, particularly in relation to the kinetics of decomposition. They proposed the relationship:

$$\log (p/p_f - p) = kt + \text{constant} \quad \ldots\ldots\ldots (1)$$

where p = pressure of gas liberated at time t after the commencement of the reaction, and p_f = final pressure attained after the end point of the reaction had been reached. They were able to provide a theoretical interpretation for this equation. Bircumshaw and Edwards (1950) have studied the rate of decomposition of nickel formate. They showed that the surface area of the material exposed, the accumulation of gaseous by-products, the preliminary treatment, i.e. whether it was finely ground or not, mixing of the salt with nickel residue from a previous decomposition, and the interruption of the heating programme all affected the rate and mode of decomposition. The decomposition followed the Prout-Tompkins relationship if the material was spread out over a large area. The solid residue catalysed the reaction and the gaseous products present at the scene of decomposition inhibited the reaction. They showed that water vapour had a particular importance in this respect, especially in connection with the induction period which was observed in almost all of the compounds studied and in the initial stages of the reaction. These workers and others have found that at constant temperature heterogeneous reactions of the type given in equation 2 proceed autocatalytically.

$$\text{solid}_1 \longrightarrow \text{solid}_2 + \text{gas} \quad \ldots\ldots\ldots (2)$$

Basalo and Castor (1953) have used some of these assumptions in kinetic studies of some olation—condensation reactions involved in hydrate decompositions. These workers and Giaque (1949) have used kinetic methods of thermal study to show that standard thermodynamical methods for characterizing solid phases in systems are inadequate.

The effect of surface area upon the catalytic effect of substances has been studied thermogravimetrically by Goodman and Gregg (1960). In this way it is possible to assess the best conditions for the production of catalysts of high activity. Tovmas'yan (1956) has published a review of the various applications of thermogravimetric analysis to physical chemistry.

Applications in Inorganic Chemistry

The technique of thermogravimetric analysis has, until recently, not been widely recognized as one which can be applied to this field. Early work has been concerned mainly with mineral chemistry. For instance Jaffray (1948) has used thermogravimetric studies to give information relating to the composition, nature, and formation of clays and minerals. It has also been applied in studies on corrosion, oxidation, and reduction of metals and alloys. The direct applications to inorganic chemistry are (i) study of solid-solid reactions (ii) study of preparations by application of heat (iii) modes of decomposition of materials (iv) studies of the behaviour of oxidising agents and (v) sublimation studies. The technique used alone may not provide a great deal of information but used in conjuction with infra-red spectroscopy, x-ray crystallography, differential thermal analysis, and other physical methods the course of a reaction or the behaviour of a particular compound may be evaluated. These extensions will be discussed in the next article.

Studies of inorganic compounds are usually concerned with the investigation of the nature, decomposition, and structure of these materials by observation of the following properties: (*a*) loss of constituent water molecules, (*b*) loss of volatile ligands in complexes, (*c*) breakdown by pyrolysis, i.e. using high temperature studies. Thus it is possible to assess the mode by which water molecules are held in the compound under study. Studies have indicated that thermal decomposition of inorganic compounds is dependent both on the nature of the anion and cation. Certain homologous series of inorganic complexes have been studied. For example complex compounds of the type $[M(X)_n Y_x]$ may be studied (i) by keeping X and Y constant and varying M, a procedure employed by Wendlandt and Horton (1960) who have studied various transition metal complexes with ethylene diamine tetraacetic acid both by thermogravimetric analysis and differential thermal analysis, (ii) by using different ligand groups X whilst keeping M and Y the same, in this way ligands such as NH_3, H_2O, en, pn, etc., may be studied. Such a study has been carried out by Borrel and Paris (1951). (iii) by using different anions whilst keeping M and X constant. This is particularly important since the application of

heat is one of the methods for the introduction of an anion into the co-ordination sphere of the metal. Work is at present being carried out by Chong, Redfern and Salmon (1960) and the authors on the systems $[Cr(H_2O)_6]^{3+}$, $[Cr(NH_3)_6]^{3+}$, and $[Cr(en)_3]^{3+}$ with a range of anions. It is often helpful in such studies to be able to control the atmosphere in which decomposition is taking place and to collect samples from such decompositions. Where such an investigation is carried out with the object of preparing new compounds, the results of the thermogravimetric study must be correlated with analysis, infra-red, and other physical methods in order to confirm the results.

Duval and co-workers (1953) have studied a series of oxidizing agents such as nitrates, chlorates, bromates, iodates, chromates, and stannates which might be expected to liberate oxygen at specific temperatures. In some instances it has been shown that weight gains up to 20 per cent have been recorded. The formation of peroxides and various unstable per-salts may be studied in this way.

Applications in Organic Chemistry

There is as yet little literature on the application of the thermobalance to this branch of chemistry. Possible applications include (*a*) studies of the thermal behaviour of homologous series, (*b*) solid-solid reactions, (*c*) thermal decomposition of organic materials in a variety of atmospheres, (*d*) study of the behaviour of isomers, and (*e*) high temperature pyrolysis. Certain organic polymers, mixtures and compounds have been studied by high temperature pyrolysis methods.

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(To be continued).

EXHIBITION LECTURES

It is hoped to start publication of the lectures given at this year's Laboratory Apparatus and Materials Exhibition in the September issue of Laboratory Practice

Physics Research

Pioneering research in a number of important branches of physics is reported in the National Physical Laboratory's report for 1960.*

Proposals for two new research programmes have also arisen during the year.

One is for a research and development programme on high speed digital computers to be carried out as a co-operative effort by government laboratories and industry. The N.P.L., who are already carrying out research into computing elements, would have a major part to play in this programme.

The second is a proposal which the Executive Committee has put forward for a research reactor, to be sited at Teddington, for standards work and for basic research by both the N.P.L. and the National Chemical Laboratory. The Committee wish to build up the general standards work for radiology and consider that a research reactor is an important facility for this. Since the Report was prepared, however, it has been decided to alter the priorities in the purchase of machines needed for this work. Although a research reactor will be needed in due course, it has now been agreed that a more urgent requirement is a Van de Graaff electrostatic generator.

A combination of pressures up to 100,000 atmospheres and high temperatures can cause profound and permanent changes in the properties of certain substances.

It is indeed possible to change markedly some of the physical properties of materials, such as the electrical conductivity, and to synthesize new materials. Using an ultra-high pressure apparatus built at N.P.L. to a modified American design, the Basic Physics Division has already carried out studies of the effect of pressure on the resistivity of semi-conductors, and produced coesite, a dense form of silica, and some very small artificial diamonds.

Polymers are widely used as plastics, synthetic rubbers and fibres and it is of great practical importance to discover the relationship between the structure of their long chain molecules and their electrical and mechanical properties. Work has continued during the year on measuring the dielectric properties and the low frequency dynamic properties of polymers.

Plans to expand considerably the present research programme in view of the mounting national concern about noise are announced by the Executive Committee. Meanwhile subjective tests on motor vehicle noise are being made in the Applied Physics Division in collaboration with the Ministry of Transport, aimed at designing an instrument which will give a physical measurement of subjectively-assessed noise for a wide range of vehicles.

A crucial experiment in the Light Division was the attempt to measure light as a 'visually weighted' radiation. This is radiation which has been passed through a filter transmitting, at every wavelength, a fraction proportional to the sensitivity of the eye at that wavelength. First results were very encouraging and aroused considerable international interest. The Division will now redetermine the N.P.L. radiation scale, after which they may be able to propose the application of an absolute radiometric method to the measurement of light.

The new programme of the reorganized High Temperature Materials Laboratory will include a greater concentration on new materials of very high melting point. The first experiments will be on the materials for the first of the new power stations using supercritical steam and later on the more advanced supercritical stations that will follow.

Work in ferrous metallurgy by the Metallurgy Division using the electron microscope, continues to make progress, and will have many industrial applications.

*Report of the National Physical Laboratory for 1960, published for D.S.I.R. by H.M.S.O., price 9s. 6d.

VARIATIONS AND ERRORS IN EXPERIMENTAL INVESTIGATIONS

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PART V—PERSONAL AND CHARACTERISTIC ERRORS

EXPERIMENTAL investigations are planned and executed by human beings who often suffer from some defect or characteristic which is associated with some degree of experimental error which is sometimes recognized as 'Personal Error.' The term is rather a loose one and covers errors associated with the person carrying out the work rather with than errors directly concerned with the equipment itself. However, the basic control of the equipment by the human makes for the experimental error which sometimes cannot be classified directly.

'Personal Errors' can often be traced to various causes, the most prominent of which are: carelessness, forgetfulness, complacency, unawareness, idiosyncracy, lack of experience, inappreciation, ignorance, lack of concentration and inattention to details.

Some individuals are more 'error prone' than others, just as some are more 'accident prone' than others. It should also be remembered that the probability of error associated with a given individual depends on certain conditions. This increases considerably when the mental state of the individual is greatly affected. Thus, personal worry, irritation, agitation, joy and elation (whether psychological or alcoholic) result in increased probability of 'personal error'. This happens sometimes without the individual appreciating the effect and results in the variation being attributed to any cause other than the personal one. The attempts of the individual to 'explain' the variations noted are similar to those advanced by the car driver who has had an accident after having 'one more for the road'.

Consider in this connection the effect of lack of concentration on the probability of error. Many experimental investigations require a complicated series of experimental procedures each of which must be carried out meticulously. In order to do this the experimentalist must be able to concentrate on the work in hand, otherwise misleading results may be obtained. This may be appreciated by considering a simple investigation into the change of absorption spectrum with pH. The experimental procedure may be classified as:

(*a*) Preparation of the correct solution and the 'blank' or control solution.

(*b*) Thermostating of the solutions.

(*c*)

pH Measurements	*Absorptiometric Measurements*
1. Standardization of the glass electrode with two buffers, say, phthalate and borate, using the sequence phthalate, borate, phthalate, borate for a check.	(i) Meticulous cleaning of the absorption cells and drying of the outside surfaces.
2. Measurement of the pH of the correct solution and that of the corresponding 'blank' or reference solution.	(ii) Standardization of the instrument (including balancing of the photocells, obtaining a good baseline in recording instruments, etc.).
	(iii) Measurement or recording of the optical density of the solution.

(*d*) Writing down of the relevant information in the notebook (e.g. time, temperature, age of solutions).

Any of these stages may be carried out too rapidly, and some may be inadvertently missed out if the experimenter's attention is not fully focused on the work in hand. Often the mistake is recognized rather late, when the damage may be substantial. Worse still the experimenter may not have realized his error or omission at all, and a significant 'experimental error' will then have been noted.

Some personal and related experiences may be quoted in this connection. A research student used to prepare his experimental solutions first and then store them in amber bottles; the 'blank' solutions were subsequently prepared and then stored in transparent bottles, so as to decrease the chances of confusing the 'experimental' and the 'blank.' One day he was rather worried about some personal matter, but nevertheless proceeded to carry out his work, apparently with the same degree of care as was his usual custom. He carried out his pH measurements without any trouble, but his absorptiometric measurements were obviously topsy-turvy. On rechecking his solutions he found that he had stored his 'experimental' solution in the transparent bottle and not in the amber one, as was his custom, but because of his regular habits carried out the measurements as if his usual storage arrangements still applied.

On another occasion a seasoned investigator received some exceptionally good news in the morning post, and when he entered the laboratory he was quite elated. Without realizing it he added six volumes of the acid to four volumes of the base, instead of the reverse proportion, in making up the required buffer solution. As he had prepared the required solution many times previously and obtained the same pH value, and as he was in a hurry to complete the measurements (so that he could leave the laboratory early to celebrate) he neglected to carry out the pH measurements first, and proceeded to carry out the absorptiometric determinations. No shift in the absorption spectrum was noted, as compared to the results at a lower pH value, and the preliminary 'deduction' was that no acid-base group was present over the particular pH region. Normally the investigator would have calibrated the glass electrode before measuring the pH values of the experimental solution and that of the blank. As the standardization of the glass electrode had not varied for over three days, and as it was supposed to have been checked by the 'Old Man' himself early in the morning, the investigator assumed in his hurry that it was calibrated. Unfortunately, the 'Old Man' merely noted that the glass electrode was a little temperamental and made a mental note to change it next morning, as he was rather busy that day. The reading taken by the investigator was therefore erroneous; but as chance would have it it was practically the same as originally expected, thus supporting the preliminary deduction. The error was discovered during the following days, when the usual checks and rechecks were made, but not before it had caused some aggravation and perturbation and some unprintable comments about the behaviour of glass electrodes.

Cases have been recorded in which laboratory personnel, when elated or depressed, have attempted to standardize the pH meter at pH 4·00 with borate instead of phthalate, or phosphate buffer at pH 6·8, and have at first complained that the pH meter was behaving erroneously. The simplest method of preventing such mistakes is to relate these stories to members of the laboratory who are then on their guard. In this connection one cannot emphasize too often the need to check both the pH of the experimental solution (containing the buffer plus solute) and that of the reference (i.e. buffer alone). Cases have been recorded in this laboratory involving pH variations as high as 0·2. (The explanation for these will be considered elsewhere).

Other cases where considerable deviations from the normal mental state have caused the instrument operators to try to measure optical transmission under the heading of absorption, or to balance the wrong photoelectric cells, or to record base-lines at more than 10 per cent optical density, have been encountered in a number of laboratories. Prevention is considerably enhanced by relating the stories because human beings naturally try to protect themselves from being laughed at after the event.

The incidence of individual 'personal errors' is affected by the presence of other persons. Incompatibility of certain people has been experienced in a number of laboratories, as well as in other walks of life, and often results in undesirable consequences, one of which is increased incidence of 'personal errors.' One example is that of two independent investigators who worked in different laboratories in the same institution. Their individual experimental results were more or less reproducible. The two got on comparatively well in the social gatherings of the institution. Owing to laboratory space redistribution it became necessary to move them into the same, sufficiently large, laboratory which had however only one laboratory assistant whom they were to share. It soon became clear that occupation of the same laboratory by the two investigators was associated with worse troubles than are encounterable when two women share the same kitchen. Further, a remarkable increase in what they called 'experimental variations' was soon noted in their individual results. Most of these were seriously considered by the two investigators to be 'biological variations,' as they worked with animals. It has been said, however, that the director of the institution wrote to the Editor of a well known encyclopaedia suggesting that the meaning of the term 'biological variations' should be expanded.

Many modern conditions are conducive to human strains and stresses and thus tend to increase the incidence of personal errors. The effort and the need to obtain more and more data adds to these stresses and is likely therefore to have undesirable consequences. Boredom with routine procedures adds its toll as impatience, often results in lack of care and inattention to details. It is in this connection that the extensive use of automation techniques, such as pH recording and recording spectrophotometry, is likely to result in decreased incidence of 'personal errors.' However, this neither eliminates 'personal errors' nor does it necessarily reduce the magnitude of the overall 'experimental error.' Consider in this connection pH values obtained in the titration of a weak acid with sodium hydroxide, using glass electrode pH measurements. The measurements may be carried out manually or using an automatic pH titrator and recorder. With both methods the calibration of the glass electrode must be carried out manually, though in the latter case the result is recorded automatically. Many more titrations can be carried out within a given time, particularly if they are repetitive. Possible personal errors, such as incorrect figures being noted or the erroneous reading of a burette, are eliminated. However, other personal errors such as the addition of an incorrect volume of the acid, incorrect initial setting of the apparatus, and incorrect reading of the chart are not only possible but have been noted. Further, the possible characteristic apparatus error is always present. For example, the recorder chart has 0·005 pH scale divisions which may be read to within 0·002 pH units. However, the temperamental behaviour of the particular glass electrode is commonly such as to involve an error of $\pm 0{\cdot}005$ pH units during the

calibration itself. Indeed, variations as high as 0·02 pH units following a complete titration are quite common. One should not be misled by the claims issued by some firms which imply a stability better than 0·01 pH units over 24 hours. Such stability is sometimes possible when the glass electrode used is rechecked with the same solution throughout. However, it is the experience of the author that the best commercial glass electrodes available can be temperamental. They may behave 'ideally' for two to three days, this being the exception rather than the rule, but they may then alter by as much as 0·02 pH units in the next six hours. Worse behaviour than this is also known; the incidence depends to some extent on the investigator, as will be seen from the behaviour of glass electrodes issued to different laboratory operators who were carrying out measurements at 40°C.

One person, A, was a creature of habit. His first action on entering the laboratory was to introduce his glass electrode into the particular thermojacket at the required temperature and to switch on the pH meter he used for the determinations of certain equilibria, irrespective of whether he was to start his experimental runs within half-an-hour or two hours. B would remember to carry out the above preparations only when he was within a quarter of an hour of starting, while C kept his glass electrode in the thermostat, at 40°C., so as to have it in continuous readiness. As was to be expected, some 'personal' variations were eventually noted. For the first three months, C had the least number of initial variations, but thereafter he had consistent trouble with his glass electrode, while the others had hardly any trouble. Apparently the continuous immersion of the glass electrode at the comparatively high temperature of 40°C. caused some wear and tear, possibly because of the higher rate of solution of the glass. On the other hand, B had a number initial variations which he hopefully described as 'induction periods.' (Needless to say he was disabused of the idea quite quickly). A had the least trouble, but because he was desirous of putting up a record of having the longest 'useful-life' glass electrode (longer than 18 months) subsequently wasted some time on treating and recleaning it, when to everybody else it was quite obvious that it should have been discarded.

The accumulation of 'personal errors' have been noted on special occasions. This can be attributed to the higher tensions and excitement which accompany the preparations for a major event. Many a supervisor can relate experiences in which a particular instrument or apparatus which had behaved perfectly normally on all previous occasions, became temperamental or undemonstrable at all. More often than not it is the personal factor rather than the fault of the instrument which is the major cause. For example, on a certain important occasion, the high voltage paper electrophoresis of amino acids was to be demonstrated. Unfortunately after the experimental run the presence of two amino acids could not be determined at all. After checks and rechecks, it was found that the particular amino acid bottles that should have contained the amino acids contained only distilled water. It was subsequently discovered that some zealous person had decided to wash all the reagent bottles and refill them in readiness for the special occasion. He managed to get through practically all the bottles but failed to wash out the distilled water and replace it with the correct solutions in those two bottles.

We have seen that tension, excitement, worry and depression can increase the incidence of 'personal errors'. From this standpoint it is desirable to have for experimental work persons with steady temperament which is only slightly, if at all, affected by the above factors. Yet it is a fact that many major scientific ideas have been conceived and developed because of times of stress. The explanation for this paradox is probably that scientific development is the result of human activity and that such activity depends on the particular person and on his temperament. Some scientist need opposition or stress to stimulate and galvanize them into mental activity. Others can only do their best when left in peace. In practice this does not detract from the overall need for undivided attention to experimental detail. Without this a multitude of experimental variations is likely to be encountered and possibly classified under all sorts of headings bar that of personal responsibility. One has only to hear some of the excuses made by some students for inefficient experimental work, caused by various diversions, to realize how many scapegoats one may find in scientific work.

We have considered here only a few of the causes which can lead to personal or characteristic errors. The list is long, and it would require a book to consider in detail the various causes and methods of dealing with them. It is hoped however that the attention which has been drawn to some of them in this paper will help in the search for others, and thus assist in reducing their incidence or even in their elimination.

The Henry Dale Professorship

The Council of the Royal Society at its meeting on May 18 warmly accepted a generous gift of £100,000 from the Trustees of the late Sir Henry Wellcome to establish and endow a Royal Society Professorship in Medical Research to be known as The Henry Dale Professorship. The Wellcome Trustees have made this gift to commemorate the unique services of Sir Henry Dale, O.M., G.B.E., F.R.S., to their Trust as its Chairman for 22 years as well as his outstanding contributions to science and medicine in a wider context. In accepting the gift the Society expresses its gratitude and great satisfaction in being associated with the Trustees in this act of honouring its distinguished Fellow, Sir Henry Dale, who has also rendered conspicuous service to the Society both as Secretary (1925-35) and as President (1940-45).

The Council of the Royal Society greatly values this outstanding benefaction which will significantly increase the Society's capacity to promote medical research.

This announcement was made on June 9, Sir Henry Dale's 86th birthday.

ELECTRONICS FOR BIOLOGISTS

by G. C. Ware

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PART VII

Practical Selection of Valves

It would be impracticable and probably impossible to describe the many hundreds of available valves and their possible applications, but as most practical problems start with the question, 'Is there an ideal valve for this application?', and invariably finish with the answer 'No,' it will be as well to examine a few of the criteria which determine the selection of a valve. Many of the available triodes and pentodes are designed for special purposes and may often be ruled out of consideration at once. Others are too large, requiring a trolley to move them and often an elaborate water supply to cool them, or too expensive, being experimental types. Yet others are obsolete or obsolescent. Ultimately, however, there will remain a number of possible valves from which to choose, whether a triode, pentode, heptode, diode or hexode is required. Perhaps the first consideration is size, both electrical and physical. The valve must be large enough to handle the proposed anode current, yet, other things being equal of as small physical dimensions as possible, as space is limited in any piece of equipment. Secondly the valve selected should have the same heater voltage as other valves it is proposed to use, and lastly must have the right characteristics for the job.

A further slight complication arises because many valves are manufactured to almost identical specifications by different manufacturers; for example the Brimar type 6C4 and Mullard type EC 90 are described as *Equivalent types*. There is usually no significant difference between equivalent types.

In the construction of amplifiers half wave rectifier valves (diodes) and full wave rectifiers (double diodes), triodes, pentodes, and beam tetrodes (very similar to pentodes) are the valves of chief interest. In general, mains equipment uses indirectly heated valves, whilst battery equipment is more economical if designed with directly heated cathodes, steady d.c. being readily available from the batteries.

For mains equipment the choice in rectifiers is between direct or indirect heating and full or half wave rectification. Where fully smoothed d.c. is required it can obviously be more easily obtained from full than from half wave rectification, though the latter often suffices for less critical demands. Direct heating of the cathode is very rapid and a supply of d.c. is provided before the other (indirectly heated) cathodes are hot; this will throw the full peak voltage on the smoothing capacitors. With an indirectly heated rectifier the amplifier circuit begins to draw current at much the same time as the rectifier makes it available. Maximum rectified current (anode current) and maximum voltage are the only other special factors to be considered.

Triodes and pentodes may be roughly divided into four classes by two divisions. Firstly into high and low slopes (high μ and low μ) depending upon whether the change of anode current for a given grid voltage change is large or small, and in consequence whether the amplification or *stage gain* is expected to be large or small. Secondly these valves may be designed to act primarily as either voltage amplifiers or as power valves. The former allow small anode currents, the latter larger ones. However, most voltage amplifiers are high slope valves whilst power valves usually have low amplification though there are exceptions.

Economy of space and heater current requirements has been achieved by the manufacture of double and triple valves which consist of two or three distinct valves in one envelope. The valves may be completely electrically separate (except for the heater) or they may have certain electrodes in common.

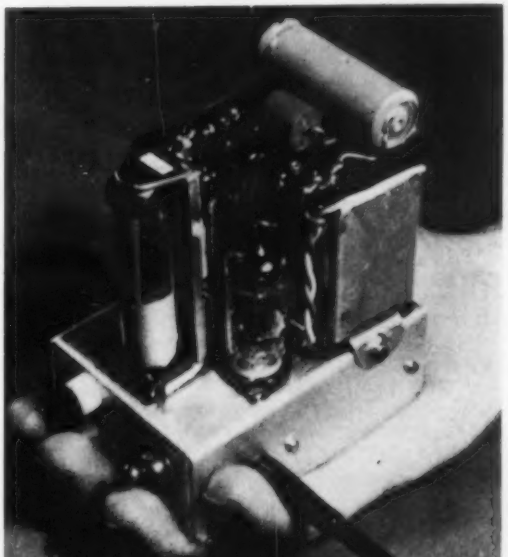

Plate IV. **The completed proximity switch. The rectifier and smoothing capacitor are mounted on top of the transformer for compactness. The relay is towards the front of the chassis and the oscillator valve has been provided with a metal cover (back-left) to reduce radiation and possible radio interference from the oscillator circuit.**

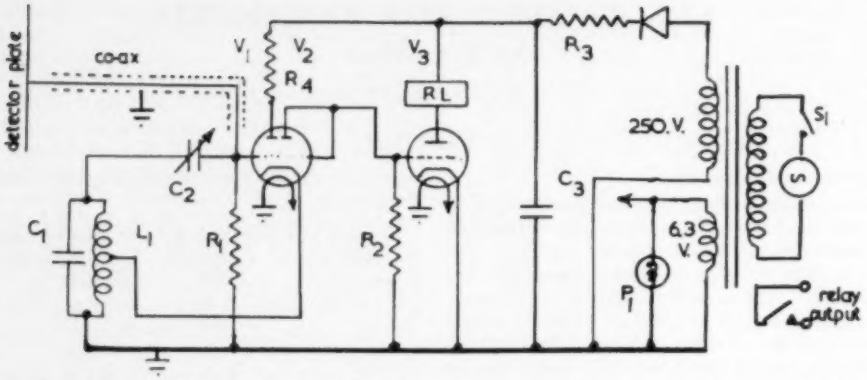

Fig. 49. A circuit for a proximity switch. The relay RL can be arranged to open or close the output circuit when an object approaches the detector plate.

Components are: C_1 = 10 pF. ceramic 1,000 V.; C_2 = 50 pF. variable air spaced trimmer; C_3 = 8 mF. electrolytic 300 V.; R_1 = 1 MΩ. ¼ W.; R_2 = 100 KΩ. ¼ W.; R_3 = 100 Ω. ¼ W.; R_4 = 100 KΩ. ¼ W.; L = 150 turns 36 S.W.G. enamelled copper wire on ¾ inch dia. former centre tapped, or Wearite Type PO1; RL = 10,000 Ω. GPO Type 600; P1 = 6·3 V, O·3 A pilot lamps; S1 = mains on/off switch; $V_{1\&2}$ = 6J6 Brimar (ECC91 Mullard equivalent type); V_3 = 6C4 Brimar (EC90 Mullard equivalent type); Rectifier = Type REC 51 Radiospare Ltd., W.1.; Transformer = Type 250 V, Midget mains Radiospare Ltd., W.1.

Design and Construction of a Proximity Switch

As an exercise it is proposed to describe the design operation and construction of an electronic proximity switch. The switch is intended to operate any electrical device when the hand, or any other object, approaches but does not touch the switch. Booby trapping, counting and the control of fluid levels are obvious examples of application. Light beams have frequently been used for switching purposes but are invariably detectable to the observer and subject to effects of sudden changes in daylight intensity and so forth. The switch described here (Fig. 49 and Plate IV) depends upon a delicately poised oscillator ceasing to oscillate when part of the feedback voltage is diverted to earth.

The Circuit

Valve rectifiers have already been adequately discussed and as the power requirements of this device are not critical (in fact the circuit would operate on a.c.), a half wave metal rectifier has been used with only one smoothing capacitor. The oscillator also has been designed rather differently from that described in the text partly for experience and partly because tuned anode circuits tend to be rather too stable for this application. The input (L_1 C_1 Fig. 49) to the grid of the oscillator forms the tuned circuit. At frequencies other than the tuned frequency the impedance will be small and the grid signal returned to earth. At the tuned frequency, the impedance will be high and the signal applied to the grid. Feedback to the grid circuit may be taken from the anode circuit as before (Fig. 50a) or from the cathode circuit in which the same signal is present. A separate coil connected between cathode and earth could be used (Fig. 50b) but the phase relationship will allow part of the coil L_1 to be used to induce the feedback (Fig. 50c).

The grid voltage is kept negative as before by C_2 but if C_2 is too small, insufficient signal will be returned to the grid and oscillation will cease. For our purposes only just sufficient signal should be returned to the grid to maintain oscillation. A variable capacitor at C_2 will allow the capacity to be reduced until oscillations are just maintained. Suppose now a capacitor is connected between grid and earth in order to divert some signal to earth. As the grid signal was already only just sufficient oscillation will cease. A small capacitor is formed by a small metal plate or wire and any other object connected to earth, such as the hand. (Fig. 51). This circuit therefore forms an oscillator which when correctly adjusted will cease to oscillate when an object approaches the half capacitor formed by the detector electrode P1.

The signal voltage generated by V_1 may be rectified

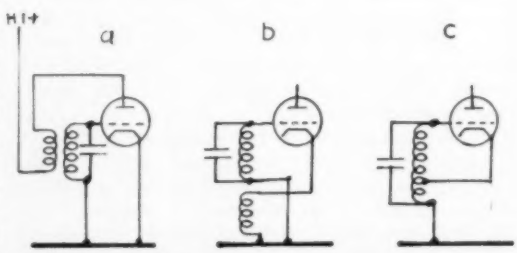

Fig. 50. Three methods of feedback in a tuned grid oscillator.
(a) From a separate coil in the anode circuit. The phase of feedback will be determined by the relative rotation of the coils.
(b) From a separate coil in the cathode circuit. The phase of feedback will be determined as in (a).
(c) Combination of the two coils in b to form one coil with a tapping for the cathode connection. This circuit will give positive feedback.

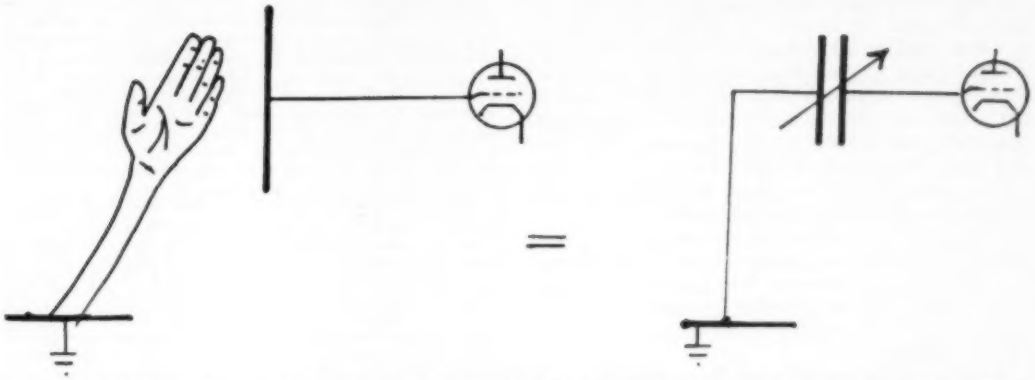

Fig. 51. An object at earth potential brought near the detector plate forms a capacitor between grid and earth and allows sufficient signal to be lost to earth for oscillation to cease.

using any conventional rectifier, and applied to the grid of another triode (V_3), to provide a large negative bias so that the anode current in V_3 is almost zero. If oscillation ceases, the signal will disappear, the grid of V_3 will return to zero voltage and the anode current will flow in the V_3. This current is made to operate a relay RL 1. and so switch on or off any desired device. In this circuit the second triode in the first valve (a double triode) is used as a rectifier. The grid is connected to the anode and the valve thereby converted to a diode. The two valves have a common cathode which forms a convenient connection for the signal which is in effect taken from the cathode of V_1 and applied to the cathode of V_2 by a ready made internal connection. Many alternative designs might be equally satisfactory, for example the signal could be taken from the anode circuit via a coupling capacitor to a metal junction rectifier and thence to the grid of V_3. In this case no coupling capacitor is required as the cathodes of V_1 and V_2 and the grid of V_3 are all at zero volts in the absence of a signal from V1.

Construction

Although the circuit is not critical the best results will only be obtained if the *total capacity* of the circuit associated with V_1 is small. The approach of an object will then produce the largest possible percentage change of capacity to operate the switch.

Before any electronic device is constructed in its final form it is essential to construct a *hook-up* to check that the proposed circuit works. The hook-up is constructed on any suitable chassis, little attention being paid to neatness and appearance. The author keeps an old chassis with a multitude of various sized holes in it for this purpose. In a hook-up the connecting wires are made relatively long to enable easy insertion of meters and additional components but as a result the circuit is invariably prone to stray capacity and hum, but some idea of the potentialities, current demands and snags of a circuit can be obtained before considerable time is spent on final construction. Plate V shows a hook up of the proximity switch with the oscillator waveform displayed on the oscilloscope. It enabled several different coils to be tried out and the best results were obtained with a hand wound coil consisting of 150 turns of 36 S.W.G. enamelled wire on a $\frac{3}{4}$ in. diameter former. The coil was centre tapped at 75 turns for the cathode connection. A commercial coil (Wearite PO.1) also gave quite good results. Coil design for this type of circuit is largely trial and error!

A low slope double triode was chosen for the oscillator and rectifier and the same type of valve in single form for switching.

It is desirable to wire a hook-up and finally the actual apparatus in logical sequence testing each portion as it is constructed. For example:

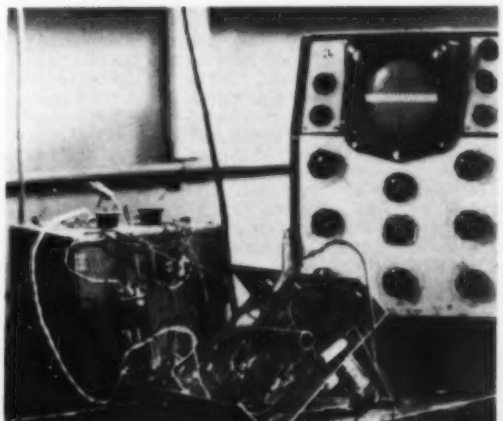

Plate V. **The author's 'hook-up' of the proximity switch being checked with an oscilloscope. The wave form on the ocilloscope. is from the oscillator in the switch.**

1. Wire mains transformer and test various a.c. outputs with meter. Connect 6·3 V. heater supply one side of which may be earthed and the chassis used as the return conductor. This procedure should not be used with high gain amplifiers as the current flowing through the chassis may produce hum; in fact with amplifiers, great care must be taken in siting the mains transformer and heater wiring to avoid hum.

2. Connect rectifier and check d.c. output polarity before connecting smoothing capacitors.

3. Connect the oscillator circuit around V1. Check, preferably with the oscilloscope. Alternatively, measure a.c. signal voltage with a suitable meter and capacitor. If the circuit does not oscillate, increase the value of the capacitor C_2 by adding a fixed capacitor of similar value in parallel.

4. Insert milliammeter in anode, or cathode lead of 6C4 and observe fall in current when V_2 anode is connected to V_3 grid. Replace meter by relay and confirm that this also operates.

5. Adjust C_2 so that relay just fails to operate. Check the effect of placing the finger near the grid connection to V_1 or the special detector plate.

6. The whole apparatus can now be rebuilt in its final form (Plate IV). A short piece of low capacitance co-axial cable may be used to join the detector plate to the switch, the outside conductor being earthed.

Water Pollution Research

The Water Pollution Research Board, in their annual report* conclude that the matters of greatest importance for the programme of research of the Laboratory at the present time are the study of the effects of polluting substances in streams and the investigation and improvement of the basic processes common to the treatment of sewage and of many industrial wastes. These basic processes include sedimentation of suspended solids, the dewatering of the resulting sludge, and the treatment of the liquid portion of the waste by biological methods; consequently these lines of research now take up a large proportion of the resources of the Laboratory.

Sewage and Industrial Waste Treatment.—Among the varied investigations of processes used in the treatment of sewage and industrial effluents, two are perhaps of particular importance. The first is a comparison of several types of media in large pilot-scale percolating filters treating Stevenage domestic sewage. During the year the installation operated without difficulty from ponding, the filters treating the sewage at a rate of 100 gallons per cubic yard per day, equivalent to the rather high loading of about 0·32 lb. B.O.D. per cubic yard per day. There are four types of filtering material, each in two sizes—1 in. and 2½ in. gauge—and there has been a very wide difference between their performance. For example, over a period of 12 months the average B.O.D. of the settled effluent from the least efficient medium—1 in. slag—was only 11 p.p.m. From February to April, when the effluents were at their worst, the effluent from the large rounded gravel contained practically no nitrate, whereas with the small slag about 90 per cent of the ammonia present had been nitrified. There were wide differences too between the proportions of anionic detergent removed by the different filters. These differences are shown to be related to the surface area of a given volume of the filtering material.

*Water Pollution Research 1960, published for D.S.I.R. by H.M.S.O., price 7s.

Detergent Trials.—Further work is reported on the progress of the large-scale trials at Luton of a new detergent containing a form of surface-active material more easily decomposed by bacteria at a sewage works than was the material at first used. As a result, the concentration of the surface-active material discharged in effluent from Luton decreased by more than half between 1958 and 1960. Numerous samples of new types of surface-active materials, undergoing development by manufacturers, have been received during the past year and have been tested for susceptibility to biological attack.

Effects of Pollution on Fish.—A wide variety of work on the effects of polluting substances on fish is reported. The most complete investigation was of the effects on trout of china clay in certain Cornish streams. It has been shown in the Laboratory that concentrations of china clay, usually between about 100 and 300 p.p.m., would kill many fish exposed to them for a period of a few months. The clay often causes a characteristic thickening, fusion, and general degeneration of the gills. Observations were made in Cornwall in clean streams and in some containing china clay, which is often present in a concentration of some thousands of parts per million. These streams contain trout, but in only about one-seventh of the numbers found in the same area of a clean river. It is not known how long they had lived there since usually they would have been able to swim into the turbid parts of the river from clean head waters. The clay greatly reduces the amount of fish food on the bottom but the trout present were well nourished and had been living to a large extent on terrestrial invertebrate animals which had fallen into the water and been carried downstream. Some of the fish caught had the same degenerative condition of the gills as was found in laboratory experiments.

Instrumentation.—A section of the Report deals with the development of instruments and of methods of physical, chemical and radiochemical analysis. The W.P.R.L. suspended-solids recorder has undergone further development and has been used for monitoring the concentration of humus entering and leaving a humus tank. Another form of this device is a simple sludge detector which when lowered into a sedimentation tank indicates accurately the level of the sludge layer on the bottom.

The Fundamental Aspects of Gas Chromatography

(*Continued from page 551*)

the concentration of solute at any point within the tail (x_1x_2f) can be calculated, and also it can be easily shown that the tail is of finite length.

We can furthermore calculate the total width (x_{2f} $-x_1$), the maximum equilibrium concentration (c_{2f}) at the front edge of the tail, and the volume of the mobile phase passed until the front edge of the tail emerges out of the column. The calculations are rather involved and will not be dealt with in the present article.

For the systems with negative b, the situation is completely reversed. The shape of the band is shown in Fig. 6; the rear of the band is sharp while the front is drawn out ahead.

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METHODS FOR THE EVALUATION OF THE ANTI-BACTERIAL ACTIVITY OF SURFACE ACTIVE COMPOUNDS: TECHNICAL ASPECTS OF THE PROBLEM

by J. G. Davis

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2. Types of surface active germicides. (a) Cationic germicides: (i) Historical, (ii) Physical properties; (b) Anionic germicides; (c) Non-ionic germicides; (d) Amphoteric, ampholytic germicides.

3. Types of test for antibacterial activity. (a) Methods involving 'complete kill': (i) The phenol coefficient, (ii) Critical killing dilution; (b) Methods involving rate if kill: (i) Suspensions, (ii) Surface films; (c) Agar diffusion methods; (d) Methods involving inhibition of metabolic activities.

4. Factors affecting tests for antibacterial activity: (a) The test organism: (i) Choice, (ii) Origin and condition, (iii) The initial number of cells, (iv) The nature of the recovery medium; (b) The environment of the test organism: (i) The chemical quality of the water, (ii) The effect of pH, (iii) The degree of aeration, (iv) Temperature, (v) Organic matter; (vi) Osmotic pressure, (vii) Detergent-sanitizers; (c) Inactivation; (d) Other factors: (i) Errors in measuring, (ii) Solubility, (iii) Instability, (iv) Incompatibilities, (v) Associative effects, (vi) Mixing techniques, (vii) Contact time, (viii) Special requirements for skin disinfectants.

5. The phenol coefficient tests with QACs.

6. Reproducibility.

7. The ultimate objective.

8. Conclusions.

9. Recommendations.

10. Acknowledgment.

11. References.

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PART I

1. Introduction and Definitions

Surface active agents may be classified as (1) anionic, (2) cationic, (3) non-ionic, and (4) amphoteric or ampholytic. In our experience the first and third groups have strong detergent but negligible bactericidal powers, and the second strong bactericidal but negligible detergent powers at ordinary use dilutions (100-1,000 pm.). The fourth group has fair detergent powers. The germicidal powers have not been extensively investigated, but very strong activities have been claimed.

In the following review certain terms have been used in a particular sense, which it has been thought desirable to define. Thus a germicide is any substance or combination of substances capable of killing bacteria; the term disinfectant connotes a powerful germicide with no specific qualifications; an antiseptic is a germicide which has no irritant effect on animal tissue under specified conditions of use, and a detergent-sanitizer is a preparation having both detergent and germicidal properties under specified conditions of use. The detergent power may be due to alkalis or organic substances or both (Soc. Dairy Tech. 1959, p. 51; Neave & Hoy, 1947; Garvie & Clarke, 1955; Clegg, 1955; Schwartz *et al.* 1958).

2. Types of Surface Active Germicides

(a) *Cationic germicides*

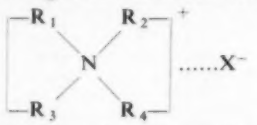
This term today usually implies a quaternary ammonium compound (QAC). For all practical purposes these compounds are non-toxic, odourless, non-corrosive, harmless to handle, sufficiently soluble, effective over a wide pH range, highly penetrative, persistent in their action and particularly effective against Gram-positive organisms. Moreover, they are non-irritant; for example, the threshold concentration causing inflammation in a rabbit's eye is about 1:330 (Davis & Resuggan, 1946).

Their disadvantages are few. They are rather expensive, less effective against Gram-negative organisms, and some types readily foam; but non-foaming types are available (Resuggan & Davis, 1947).

They are not easily rinsed away: but this persistence is advantageous and the traces remaining on food equipment are not of any public health significance.

(i) *Historical.* Hexamethylenetetramine was introduced as an internal antiseptic by Nicollaier as long ago as 1894, and Einhorn & Göttler recognized the germicidal properties of the chloroacetylaminomethanol derivative of hexamethylenetetramine in 1908. In 1913 Reychler described the synthesis and properties of cetyltriethylammonium iodide, and Jacobs (1916) and Jacobs *et al.* (1916*a*,*b*) studied the relation between the chemical constitution of the quaternary hexamethylenetetramine salts and their bactericidal properties. Little further interest was taken in these compounds, apart from Hartmann & Kägi's paper in 1928, until 1935, when Domagk emphasized the strong germicidal power of alkyldimethylbenzylammonium halides. Many papers on medical applications followed in rapid succession, and in the 1940s many papers appeared in which applications in the food industry, catering, public health, dairying, brewing and other fields were dealt with.

All QACs may be regarded as members of the group



The radicles R_1, etc., may be short, medium or long paraffin chains, or simple or substituted benzyl or heterocyclic radicles such as pyridinium. Detailed information on the many known QACs is given by Glassman (1948), and Lawrence (1950) and Resuggan (1951) have discussed their application in the food industries. The most recent collected information on these compounds is given by Reddish (1957) and Schwartz *et al.* (1958).

(ii) *Physical properties.* The QACs ionize to give a large positively charged ion and a small negatively charged hydrophilic ion. Compounds of this type have a tendency to form micelles (McBain, 1913, Ralston *et al.* 1942; Valko, 1946; Ralston 1946) and micelle formation may account for anomalous behaviour (Adam & Shute, 1938). With dilute solutions, where there is little micelle formation, the final surface tension may not be reached for a week.

It has been suggested that bactericidal power is related to surface activity (Suter, 1941; Cowles, 1938), but many surface active compounds have negligible germicidal activity. The peculiar properties of QACs and their much greater 'killing' effect against Gram-positive organisms support the theory of a specific reaction with constituents of the cell wall.

(b) *Anionic Germicides*

Soaps. Although soap alone is not generally considered a strong germicide, it is effective under certain conditions against some organisms (Walker, 1924, 1925, 1926), and a 0·25 per cent soap solution may be more effective against streptococci than 0·7 per cent Lysol or 0·1 per cent mercuric chloride (Colebrook & Maxted, 1933). However, the greater value of soap, as of all substances having detergent properties, lies in its power to remove adhering organic matter which may serve as a matrix for bacteria amounting to millions/g. and from the numerical standpoint a detergent may be more valuable in removing bacteria mechanically than an accompanying disinfectant (or disinfectant power) in killing them. However, simple detergent efficiency is difficult to measure and it is usually not possible or necessary to assess these two effects separately.

Soaps are pre-eminently of interest from the point of view of cleansing the human skin (this term covers the

removal of adhering matter, the killing of pathogens and the reduction of the bacterial numbers to a very low level), and the assessment of the usefulness of a soap for this purpose is a complicated problem (cf. Bettley, 1960).

Phenol-soap mixtures. Although soap by itself may have only weak germicidal, as distinct from detergent, properties it has the power of solubilizing all types of phenolic compounds, including the strongly germicidal cresols and chlorinated cresols (Engler and Dieckhoff, 1892; Bean and Berry, 1950; Berry, 1952; Finch, 1958). From the bacteriological point of view these mixtures may be regarded as phenols with detergent properties, and most of the tests which are suitable for measuring the activity of phenol should be suitable for them.

Other anionic compounds. The only other types of any consequence are the sulphated alkyl and sulphonated aryl compounds. Some of these may be useful against Gram-positive bacteria, but almost all are ineffective against Gram-negative types. In addition the effective pH range is limited. The members of this general group of anionic detergents are invaluable as cleaners but of little value as germicides.

(c) *Non-ionic germicides*

Although non-ionic detergents are without any significant germicidal power they can be compounded with iodine to give the iodophors, which are both surface active and powerful germicides. In the latter respect they behave similarly to iodine but without the staining and other disadvantages of that substance. They have received some attention in America (Johns, 1954) and are now being tried in this country (Cousins *et al*. 1959), particularly in the dairy industry. Both from the use and testing points of view they may be regarded as iodine. However, micelle formation may affect activity as with the QACs (cf. Allawala and Riegelman, 1953).

(d) *Amphoteric (ampholytic) germicides*

The members of the fourth group, although known chemically for many years, are relative newcomers in the disinfectant field. Strong claims have been made for their germicidal powers, but little is known of them in this country and they are not yet in general use.

One of the first workers to stress their possibilities was McCutcheon (1955), who described the properties of dodecyl-β-alanine. The ampholytics are not necessarily confined to carbon and nitrogen compounds, but only these appear to have been used so far. They are based on two main types, viz. long chain N-substituted amino acids and long chain betaines.

Solutions of the alkylaminopropionic acids may have very low surface tensions and are therefore good wetting agents. They form micelles at all pH values and are fairly good emulsifiers. Like the QACs they are strongly adsorbed at low pH values. They are compatible in all forms with anionic, non-ionic and cationic agents, with the sole exception that the acid salt is incompatible with anionics. Typical examples of these compounds with their trade names and manufacturers, are given in Table I.

Although excepted as ampholytic, the betaines are really internal QACs and Moore (1960) suggested that these should be called *intronium* surface active agents. While in some respects they resemble QACs, their general physical properties are very similar to those of the alkyl amino acids.

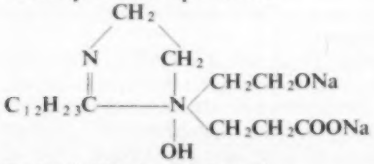
Compared with the anionics and non-ionics (which have good detergent but poor germicidal properties) and the cationics (with poor detergent but good germicidal properties), the amphoterics have good detergent and, it is claimed, good germicidal properties. The critical micelle concentration is 0·2—0·4 g/l., which may be compared with the level of 1-2 g/l. for the anionics and the cationics (Schmitz and Harris, 1958). Owing to their dipolar nature they may also form aggregates by 'internal desaturation' (McCutcheon, 1955).

3. Types of Test for Antibacterial Activity

Two of the most important methods involve determining the concentration necessary to kill a given number of cells in a given time or measuring the rate of kill by making colony counts at intervals. Another

TABLE I

Typical amphoteric (ampholytic) surface active germicidal agents

Chemical name or formula	*Trade name*	*Manufacturer*
Dodecyl-β-alanine	Deriphet	General Mills
Dodecyl-β-aminobutyric acid	Armeen Z	Armour
N-Lauryl-aminosulponic acid	Siposan	Sipon
	Miranol	Miranol Chemical
Dodecyl-di(aminoethyl)-glycine	Tego	Th. Goldschmidt

type of test is that in which a zone of inhibition of growth of the test organism is produced, and finally there are methods in which activity is measured in terms of the inhibition of some metabolic activity by the germicide. These four kinds of method are briefly considered below.

(a) *Methods involving 'complete kill'*

It is obvious that these methods are influenced by the concentration of cells in the test solution more than the methods depending on rate of kill, as the higher the initial number the longer the time or the greater the concentration required to give a 'complete kill.' But since the criterion of a 'complete kill' is usually the failure of a small volume (e.g. 1 loopful) of the mixture of cells and disinfectant to initiate growth when inoculated into a suitable medium the effect of random sampling becomes important and in practice these methods (sometimes called 'end-point' methods) probably measure something less than 100 per cent kill. The extent of the difference depends on the volume subcultured and the number of replicate tests as well as on the general conditions in the test.

In all tests where the presence of survivors is judged by the appearance of turbidity in the subcultures, care must be taken to verify that the turbidity is actually due to growth. Lower concentrations of a QAC which forms a precipitate with constituents of a culture medium may produce a turbidity when higher concentrations do not because sedimentation occurs. Also the germicide solution itself may be turbid, and soap solutions may vary in turbidity (Bean and Berry, 1951).

(i) *The phenol coefficient.* In spite of the many criticisms that have been made the phenol coefficient method (Rideal and Walker, 1903; Ruehle and Brewer, 1931; Specification, 1934; McCulloch, 1945; Reddish, 1957; Finch, 1958; Sykes, 1958) is still used all over the world for measuring the disinfectant power of germicides. It has the substantial advantage that a simple numerical value related to the behaviour of a pure chemical substance is obtained. In this method the errors associated with the type and physiological condition of the test organism are supposedly standardized, but there are important limitations to its use with QACs. These are discussed in detail in section 5.

(ii) *Critical killing dilution.* In this, the highest dilution (lowest concentration) capable of killing a suspension of bacteria under standardized conditions is determined. As with the phenol coefficient method it has the advantage that the result can be expressed as a simple and easily understood number. For cetylpyridinium chloride Quisno and Foter (1946) obtained values varying from 1:1,500 with a *Mycobacterium* sp. to 1:127,500 *with Strep. pyogenes* in the absence of serum, and from 1:1,000 to 1:20,000 respectively in the presence of 10 per cent of serum. In similar tests a number of QACs gave limiting dilutions varying from 1:1,600 to 1:200,000 (bactericidal) and from 1:4,800 to 1:800,000 (bacteriostatic) (Lawrence, 1950, p. 72). Bactericidal were distinguished from bacteriostatic effects by making transfers from the test cultures to fresh tubes or plates.

As one method of removing residual germicide Nagel (1940) washed the cells after treatment. With this technique not only staphylococci and streptococci but also coli-aerogenes bacteria were destroyed in 1 min. by 0·25 per cent QAC, and *B. subtilis* spores were killed in 15 min. by 1—2 per cent; but *M. tuberculosis* was not killed in 24 hr. by 2 per cent.

(b) *Methods involving rate of kill*

(i) *Suspensions.* The simplest basic test for disinfectants is to allow the substance to act on a suspension of bacteria under standardized conditions and to measure the rate of killing by colony counts or some other suitable method at specified time intervals. This was the method used in the classical disinfection studies (Chick, 1908, 1910, 1921; Phelps, 1911; Withell, 1942; Jordan and Jacobs, 1944, 1945). While this method will remain the choice of research workers, it is of limited value to the user who needs to know whether a germicide used as prescribed will sterilize a given piece of equipment, i.e. give a 100 per cent kill, under the worst conditions which occur in practice. However, it is in fact doubtful whether 100 per cent kill is necessary for a germicide to be effective in practice. Tests are usually made with at least 10^6 cells/ml., a far higher number than is usually encountered in practice, e.g. on detergent cleaned utensils or on washed skin. Moreover, even if a few surviving bacteria can grow under the optimum conditions provided in disinfectant tests, e.g. in broths at 37°, it does not follow that all of these would proliferate on the utensil or on the skin after removal of the germicide. This aspect is of special significance for QACs and other germicides which display a 'persistence effect.'

Finally, it is well recognized that a minimum number of viable cells, which may be relatively large, is needed to start an infection or cause a defect; the probability is that no one has ever suffered harm from a few isolated bacteria surviving from a germicidal treatment. Special cases such as that of *M. tuberculosis* are not included in this generalization as they are outside the present field of enquiry, but even here a minimum number of cells, e.g. 100, may be necessary to establish an infection.

If this approach to the problem is accepted it follows that a 99·99 per cent kill in a short time under standardized conditions can be accepted as a satisfactory criterion. This has the advantage that the required bacterial counts are conveniently made. Also, the result is not invalidated by the occurrence of the occasional and apparently inevitable 'freak survival.' If 100 per cent kill is adopted as a criterion such survivals complicate the testing.

(ii) *Surface films.* In practice it is usually the destruction of bacteria in films of organic matter on surfaces, e.g. of tissues, metal, glass and textiles, which is required and it was early realized that suspension tests, although relatively easy to perform and standardize, were of limited value in predicting the efficiency of a germicide

used for sterilizing skin, milking equipment and blankets (Clegg, 1955). Of the various factors involved, the physiological condition of the bacterial cells and the possible protection afforded are probably the most important. For example, it is much easier to kill a suspension of a 24 hr. broth culture than the bacteria in a dried milk or serum film three days old. In the latter case the cells are static and protected by a firm film of milk or serum solids. The detergent or disintegrating power of a detergent-sanitizer is then of equal importance to its germicidal power. In practice the effect produced depends on both detergency and germicidal action. Detergency is such a complicated process that no simple test is available for assessing it, but though a film test really measures two effects the method is sound from a practical point of view. Whether the bacteria attached to a surface by dried organic matter are killed or merely removed and washed away is usually immaterial in the food industry. In medicine, or when working with pathogens, the killing process can still proceed after the germicidal solution has ceased to act on the surface concerned. In surface film tests factors inoperative in suspension tests may come into play. Thus Garvie and Clarke (1955) found that humidity affected the proportion of survivors in tests with *E. coli* but not with *Staph. aureus*. More organisms survived in films than in solutions.

In the 'use-dilution' method of Mallmann and Hanes (1945) the test organisms are dried on glass rods for 30 min., exposed to a solution of the disinfectant for 1, 5, 10 and 30 min., the rods rinsed with water or an inactivating solution and then placed in culture media. This method is stated to be satisfactory for QACs. Jensen and Jensen (1933) used cover slips, and Klarmann and Wright (1946) glass slides.

A similar technique is the glass slide method of Johns (1947) in which a suspension of the test organism in 10 per cent skim milk is applied to slides, dried, immersed in the solution, rinsed and then placed in an agar medium. Stuart (1947) used glass rings.

Basically similar tests using metal equipment more closely related to practical conditions have been developed by Meewes (1941), Neave and Hoy (1947), Hoy and Clegg (1953), Clegg (1955), Garvie and Clarke (1955), Hirsch and Muras (1955), Garvie (1957) and Whitt (1958). Goetchius and Botwright (1950) used rubber strips, which are notoriously difficult to sterilize.

(c) *Agar diffusion methods*

These methods have become popular, not only as screening or sorting tests for disinfectants but also as standard methods for the assay of antibiotics. The disinfectant or antibiotic is allowed to diffuse from a cup cut in an inoculated agar plate or from a cylinder placed on its surface and produce a zone of growth inhibitation. Alternatively impregnated filter paper discs may be used (Vincent and Vincent, 1944). There is usually a linear relation between the logarithm of the concentration of disinfectant and the diameter of the zone of inhibition. Standardization of all details is essential if reasonably reproducible results are to be obtained. Thus the time and temperature at which the agar plates are hardened can have appreciable effects on the results (Davis, unpublished data).

(d) *Methods involving inhibition of metabolic activities*

Numerous methods based on the inhibition of some easily measurable metabolic activity such as respiration, reducing power or fermentation, have been published. While of considerable fundamental interest such methods are even further removed from practical applications than those depending on a mathematical treatment of survivor counts.

Some of these methods depend directly on the nature and extent of the damage done to the cell by the disinfectant. Thus in the method of Maurice (1952) the opacity changes in a mixture of cells, dye and germicide depend on the alteration of the permeability of the cells by the germicide. Manometric metabolic methods may be suitable for QACs but not for other types of germicide (Hugo, 1952).

(*To be continued*)

NOTE: References will be published with the final part of the series.

Beilby Medal and Prize, 1961

The Administrators of the Sir George Beilby Memorial Fund, representing the Royal Institute of Chemistry, the Society of Chemical Industry and the Institute of Metals, have decided to make awards from the Fund in 1961—each consisting of the newly instituted gold medal with a prize of 100 guineas—to the following:

Constantin Edeleanu, M.A., Ph.D., in recognition of his work on the corrosian of metals and alloys, with special reference to the development of the potentiostat technique and its applications to the study of practical problems. and on the characteristics of corrosion reactions in fused salts.

Prof. Jack Nutting, M.A., B.Sc., Ph.D., F.I.M., in recognition of his work in physical metallurgy, especially in the application of the electron microscope to the study of the relationship between microstructure and mechanical properties of metals and alloys and to the investigation of phase changes and dislocation interactions.

Nuclear Structure Research

The Department of Scientific and Industrial Research is to contribute over three-quarters of a million pounds towards a new nuclear physics research centre at Oxford University. This is the largest single grant ever made by the Department and will provide a particle accelerator of novel design with which Prof. Denys Wilkinson proposes to carry out an extended programme of long-term research on nuclear structure. In addition the National Institute for Research in Nuclear Science will carry out the design of part of the accelerator at a cost to the Institute of £200,000, which with further aid from the University Grants Committee, brings the total government support for the project to approximately one million pounds.

The D.S.I.R. grant will cover: a 12 MeV horizontal tandem Van de Graaff electrostatic generator; a vertical 8-10 MeV Van de Graaff electrostatic generator, and the essential buildings to house the machines.

BOOK REVIEWS

Electron Microscopy, A Handbook for Biologists, by E. H. Mercer, D.Sc., Ph.D., and M. S. C. Birbeck, M.A. Oxford: Blackwell Scientific Publications. 1961. pp. 76. 9s. 6d.

This little handbook is a practical instruction manual for biologists who use the electron microscope. It is not addressed to the electron microscope technician, but to the practising biologist preparing his specimens. It is, in fact, only a manual for specimen preparation. After a brief, and rather useless, introduction, Chapter 2 deals with the cell fixatives osmium tetroxide, formaldehyde and potassium permanganate. Fixation procedure is described in detail. After a one-page chapter on washing and dehydration, a useful number of embedding procedures are described, with much 'know how' incorporated. Then follows a section on methods of increasing contrast (so-called 'staining') by incorporating atoms of high electron scattering power. Sectioning, microtome and mounting techniques occupy a relatively fair amount of space and this is a chapter that can be read with profit by electron microscopists working in fields other than biological. A few brief notes on replication are included, surprisingly brief indeed, and this section could certainly have been extended. The effective text ends with a rather indifferent four-page section on photography. There follow 15 brief lists of formulae and operations schedules and a list of sources of supplies. There is naturally little that is original in so small a manual yet to the practising electron microscopist it is worth a purchase for the practical know-how it contains.—S. TOLANSKY.

Perchlorates, Their Properties, Manufacture and Uses, edited by Joseph C. Schumacher. ACS Monograph No. 146. New York: Reinhold Publishing Corporation, London: Chapman & Hall Ltd., 1960. pp. 257 + xii. 70s.

The opening chapter gives a brief history of the discovery and investigation of the perchlorates. The four following chapters deal with the preparation and properties of the perchlorates, from perchloric acid itself through its metallic salts, both common and rare, to a number of interesting substances, both inorganic and organic that contain the perchlorate radical.

Further chapters cover the manufacture of perchloric acid and perchlorates; the analysis of perchlorates and their use in analytical chemistry; perchlorates in explosives and propellents; the biological action of perchlorates; safety in handling perchlorates and equilibrium data on aqueous systems of perchlorates. An appendix gives statistical data on perchlorate production.

The volume is the work of six contributors. This has led to a certain amount of repetition, but such repetition is not to be disparaged when it is concerned with matters of safety. It is compiled from a reference file accumulated since 1942, and much of the less important information is in the form of more or less brief abstracts. Although the review is, on that account, somewhat sketchy in places, it appears to be reasonably comprehensive. This may be said even of those sections that deal with miscellanea, though the use of ferric perchlorate in the spectrophotometric determination of small quantities of chloride (P. W. West and H. Coll, *Anal. Chem.*, **28**, 1834 (1956)), is missing from the section on miscellaneous applications in analysis.

Certain subjects, such as the manufacture of ammonium perchlorate, are given a much fuller treatment which bears the mark of first hand knowledge. Copious references are given throughout the book to original literature. It would be unfair to highlight the few misprints and very few errors in a publication so close-packed with facts and figures, but on page 2, the word 'anhydride' is first used apparently to signify anhydrous perchloric acid and only four lines later it refers clearly to Cl_2O_7; while on page 63, 'malinate' is printed three times when 'malonate' is intended.

Any chemist who handles perchloric acid or perchlorates should equip himself with all the knowledge available on the subject, because the gaining of knowledge by experience with compounds of this sort may well lead one into a bitter or indeed fatal experience. This is certainly the book to provide the knowledge, or at least the key to it.—W. C. JOHNSON.

Sewage Treatment, Basic Principles and Trends, by R. L. Bolton, A.M.C.T., M.R.S.H., M.Inst.S.P., M.I.P.H.E. and L. Klein, M.Sc., Ph.D. (London), F.R.I.C., M.Inst.S.P. London: Butterworths, 1961. pp. vii + 161. 30s.

The authors' aim, outlined in the preface, has been to provide a thorough introduction to the principles of sewage treatment and to cater for the needs of foremen, working managers and young assistants on sewage works, public health inspectors and students of civil, municipal and sanitary engineering. In this purpose the authors have succeeded admirably. The style is lucid, the plan is simple and the text is illustrated by figures, diagrams and tables. No apology is offered, and none is needed, for the emphasis placed on the chemical basis of sewage treatment nor for the importance accorded to trade wastes. In fact chemists in industry who are concerned with the problems of effluent disposal, no less than those who earn their living on a sewage works, will find this book a very useful guide to the principles involved.

After a brief introductory chapter summarizing the main recommendations of the Royal Commission on Sewage Disposal, which form the basis of the standards of purification to which methods of treatment should conform, the composition of sewage and the main chemical changes which take place under aerobic and anaerobic conditions are discussed. Examples are given of the use of analytical results in calculating percentage purification, sludge volume, weight of press-cake and percentage reduction in organic matter during sludge

digestion. Two chapters, describing sewerage systems, storm water overflows, screening, removal and disposal of grit and removal of oil and grease, are followed by a whole chapter on primary sedimentation, in which the different designs of quiescent and of continuous-flow sedimentation tanks, and the principles of mechanical flocculation and chemical coagulation, are explained. A useful table, comparing the advantages and disadvantages of percolating filters and activated sludge treatment, tests for the quality of activated sludge and a brief account of oxidation ponds, are given in the chapter on aerobic biological treatment and, in the few pages on methods of improving final effluents, an interesting though brief account is given of recent work on micro-strainers. Seventeen pages are then devoted to a chapter on sludge disposal.

Some 20 different industrial effluents are covered, necessarily very briefly, in a chapter of 23 pages on the effects of trade wastes on sewage treatment, and there are useful formulae for calculating the population equivalent and for fixing the charges for admission of a trade waste to the sewer. The authors might well consider expanding this chapter in future editions of the book; the present account whets the appetite but tends to leave one's curiosity unsatisfied. In the final chapter, on trends in sewage treatment, a number of recent improvements are indicated. No mention is made, however, of the possibility of reducing the sludge disposal problem by designing a balanced activated sludge system which produces little or no excess sludge although, according to reports, several total oxidation plants designed on this principle are already in operation in the U.S.A.

The authors are to be congratulated on covering so much in relatively few pages. One shortcoming, in the reviewer's estimation, is the dismissal of the subject of chlorination in little more than a paragraph. Surely students of sewage treatment should know something of the factors constituting chlorine demand in sewage and in trade wastes, how it is measured and what benefits and hazards may attend the process of chlorination?—L. A. ALLEN.

Oszillographische Polarographie mit Wechselstrom, by J. Heyrovsky und R. Kalvoda, Berlin: Akademie-Verlag, 1960. pp. viii + 198. DM. 23.

The technique of oscillographic polarography using alternating currents has been developed almost entirely by Heyrovsky and his co-workers in Prague. Numerous papers have been published but, apart from a small volume by Heyrovsky and Forejt in 1953, this is the first book on the subject. Written in the German language it provides an authoritative survey and collects together the important published information. The basic principles are given and the theory of the various direct and derivative methods of working is presented. The effect of the degree of reversibility of the system studied on the oscillogram obtained is described and these and other kinetic phenomena are discussed. The effects of temperature, frequency, the capacity of the electrode system and other parameters are considered.

A general description of the electronic apparatus and some circuits are given, but these are less adequate than is desirable in view of the novelty of this technique. Details of commercially available instruments are presented.

A derivative system in which $\frac{dE}{dt}$ is plotted against E is the most useful and widely applied of the oscillopolarographic methods. This causes an elliptical trace on the cathode ray tube screen, upon which the changes in slope of the voltage-time curve at constant current, due to the oxidation or reduction of a depolarizer appear as 'cuts-in.' The depths of these 'cuts-in' are used as a quantitative measure of concentration and their shape, and the dynamic nature of the whole trace makes the method less satisfactory for quantitative than for qualitative determinations. Semi-quantitative, or even quantitative determinations are, however, often possible and sometimes valuable. Chapters are devoted to descriptions of methods of quantitative measurement of these 'cuts-in,' including a number of instrumental devices, such as the use of a double beam cathode ray tube with a calibrated Y shift on the second beam and oscillographic and comparative titrations.

Important chapters describe the oscillopolarographic behaviour of inorganic species, many organic substances and such materials as noxious gases and vapours. These are valuable because the most important application of this technique is in the rapid quantitative and sometimes semi-quantitative characterization of species, the oscillopolarographic behaviour of which is often very different from that observed by classical polarography.

This is especially true in the case of organic compounds, many of which may be characterized by oscillopolarography, whereas they do not yield a conventional polarographic step.

The final chapter gives brief details of newer instrumental devices designed to produce a standing wave on the cathode ray tube screen by methods such as the use of a synchronized dropping mercury electrode. It is probable that such developments will lead to an improvement in the quantitative aspects of the technique. A bibliography of 172 references is appended.

This book must be welcomed as an important addition to the literature of polarography, which should assist the more general understanding of the technique. The authors' enthusiasm has, however, led to the common error of making the methods appear more important and universally applicable than is likely to be the case. It is well-written and adequately illustrated and a good balance between the various topics discussed is maintained. An English translation might assist it to have the widespread reading that it deserves.—

G. F. REYNOLDS.

LABORATORY EQUIPMENT & APPLIANCES

Coulter Counter, Model 'D'

A new Coulter counter—Model 'D'—is now available which has been specially designed for the routine counting of blood cells in medical laboratories.

The new model is a similar and more compact version of the standard Coulter counter, utilizing the same basic system of passing a suspension of blood cells through a small orifice simultaneously with an electric current. The individual blood cells passing through the orifice introduce an impedance change in the orifice, and are counted at a rate in excess of 5,000 individual cells per second. The number of cells counted is approximately 100 times greater than the usual microscope count to reduce the statistical error by a factor of approximately 10 and is completed in 20 seconds.

The high degree of accuracy and reproducibility of results obtained with the instrument is matched by the simplicity and speed of its operation. A beaker, containing a suitably diluted sample of blood, is placed on a spring platform so that the orifice tube is immersed in the sample. The count is automatically made by turning a stop-cock and depressing the count switch. The count is displayed on a decade counter; in the case of red blood cells this is multiplied by 100 to give the number of cells per cubic millimetre The white cell count per cubic millimetre is the figure shown on the counter. After noting the count the beaker is removed and the next sample positioned on the stand. No rinsing or cleaning of the orifice tube is required between samples. The instrument is readily operated by a junior technician and as many as 60 samples per hour can be dealt with.

The instrument is contained in a single metal case (except for a waste bottle) measuring 13 in. by 15 in. and only requires plugging into the mains for immediate operation. It weighs 31 lb. and is easily transported from one place to another. The price is £650 complete with all accessories.

(Manufacturers: Coulter Electronics Ltd., 2-4 Ashwell Street, St. Albans, Herts.)

Model D Coulter counter.

Viscometer Bath

To meet the need for viscometer baths operating to higher temperatures, Tamson equipment is now being marketed in this country. This equipment can be used up to 230°C. with an unrivalled accuracy obtained by the use of a short period full load automatic control system.

The outside dimensions of this new bath are 35 cm. × 63 cm. and the standard unit has four 2 in. openings in the top plate (with lids) for the suspension of ASTM viscometers. The working-space is 27 × 23 × 34 cm. deep, and there are glass windows 27 × 27 cm.

When filled with water the maximum temperature variation is as low as ± 0·005°C., while with oil it is ± 0·01°C. An exceedingly high temperature accuracy is achieved throughout the working space by means of a wing-stirrer which causes rapid circulation of the liquid via circulation partitions.

There is only one control knob for switching from heating up to maximum precision, and the boost heating is automatically regulated so that no control is required even during the initial run-up period. The rate of heating is 1°C. per minute for water, and 1°C. per half-minute for oil.

The construction is to the highest possible standard, all parts in contact with the bath liquid being of stainless steel; the windows are of double walled safety glass, while both the bearings and the handles on the lids are Teflon. The specially designed immersion heaters are made from quartz and stainless steel, and a relay (provided with a rectifier and condenser) prevents sparking in the contact-thermometer, which is thus ensured of a longer life.

(Obtainable from: Camlab (Glass) Ltd., Milton Road, Cambridge.)

Electromagnetic Water Bath

This new apparatus, designed for use in blood transfusion centres and medical analytical laboratories, makes it possible to carry out all biological tests for blood coagulation with great ease and precision.

The Paris-Labo bath obviates 90 per cent of the drawbacks, due to the human element, which are inherent in other methods; it is no longer necessary to remove the glass tubes from the water bath in order to determine the exact moment at which clotting takes place and the manipulation is reduced to the barest minimum while the reading of the final result is greatly facilitated.

The electromagnetic water bath consists of a Plexiglass tank, 320 mm. long, 250 mm. wide, and 200 mm. high; over the entire length there is a 50 mm. projection beneath which is situated an electro-magnetic device which generates four magnetic fields whose intensities can be varied, and which carry out a uniform motion. Haematological tubes, placed in containers lying beneath, can be exposed to the magnetic action (which operates on the bottom of the tube through the wall of the bath) independently of one another. The tank is raised to its controlled temperature of 37°C. and the appropriate reagents are loaded into the tubes, together with a calibrated water-free indicator; the

LABORATORY APPARATUS AND MATERIALS EXHIBITION

At the request of the majority of exhibitors at the recent successful Laboratory Apparatus and Materials Exhibition at the Horticultural Hall, Westminster, London, S.W.1. next year's exhibition is to be held in the **New Exhibition Hall, Harrogate, Yorkshire, from May 14 to 18.**

In future the London show will be held in alternate years, the next being in 1963 at the Royal Horticultural Society Halls.

rotary motion of this, with the magnetic field synchronizer, ensures complete mixing of the components. The bottoms of the tubes are illuminated by tangential lighting and can be very easily observed on the black reflecting surface of the bottom of the top-projection.

At the moment of coagulation, a delicate metal pin ceases to rotate due to the change in milieu; the mobile indicator becomes motionless when the couple which causes the circular movement of this pin is exactly balanced by the resistance of the forming blood clot, and an accurate timing may be obtained.

Since the intensity of the magnetic field of force is constant, there is after every test, a measurement of the time required for the formation of a certain quality and quantity of fibrin; this is difficult to establish by normal optical observation. Furthermore, the procedure permits of a saving of time, material and reagents, since one measurement suffices for any determination, whether a normal or a pathological plasma is under investigation.

This remarkable new French equipment, first demonstrated in England at the recent Laboratory Apparatus & Materials Exhibition, is now available in this country.
(Available from: Camlab (Glass) Ltd., Milton Road, Cambridge.)

Infracord Spectrophotometer

The new model 137 Infracord spectrophotometer gives an automatic record of an infrared spectrum from 2·5 to 15μ. The standard time of 12 minutes for a complete spectrum has been carefully chosen to allow accurate quantitative measurements to be made. The new two-speed drive preserves this scanning speed but also allows fast survey spectra to be run in three minutes. These rapid scans can be used except where the highest accuracy is needed, and allow a considerable saving in the analyst's time.

A new slit system has been incorporated. This gives improved resolution, especially at short wavelengths, and in addition the slit programme may be set at a number of different levels. The narrowest programme is used where the highest resolution is required, while wider programmes allow a considerable improvement in quantitative accuracy.

The changes have also allowed a redesign of the front panel. The instrument is simply operated by three control knobs on the front panel. The mains switch and two setting-up controls are located in a small panel at the side of the instrument.
(Manufacturers: Perkin-Elmer Ltd., Beaconsfield, Bucks.)

A New Balance Case

A new balance case of basically new design and made expecially for the range of Griffin balances introduced during 1960 (though it may be used to house most other balances) is now on the market.

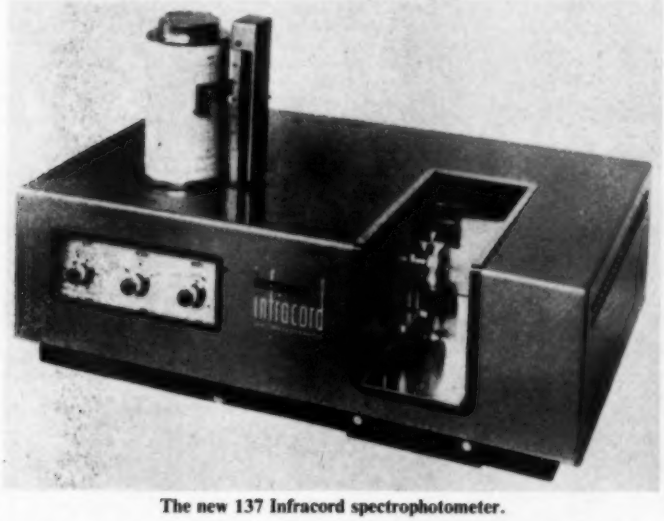
In place of the traditional wooden structure, there is a pressed aluminium sheet framework which is fabricated in three sections and then mounted on a resin-impregnated wooden base faced with an easily cleaned, attractive, linette-finish plastics surface.

There are only two glass panels, one in the front panel and the other at the rear, sealed in. The sliding front window, with its sash cord and counterbalance have vanished. Instead, across the whole front of the cage, there is a rising front panel, which has a friction hold and lifting handle enabling it to be set firmly at any height. without causing vibration. The channel holding the glass edges is air-tight while permitting the smoothest action.

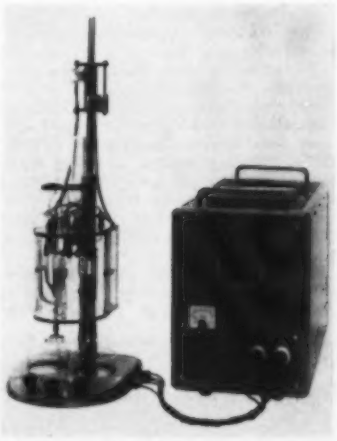
The angle of slope of the front is such that easy access for the manipulation of the riders is possible. This is particularly important for the Griffin Standard, Duorider and Chaindial balances.

The case is finished in corrosion-resistant light grey melamine based stoved enamel which gives the maximum of reflected light to the interior; full vision, once obstructed by wood framing, is given by the large glass panels in their attractive grey plastics channels. A dust-free atmosphere is always maintained when the case is closed.

Interior dimensions: 19¼ in. wide, 7½ in. deep and 16¾ in. high (49 cm. by 19 cm. by 43 cm.). Price in the United Kingdom: £4. 12s. 6d.
(Marketed by: Griffin & George (Sales) Ltd., Ealing Road, Alperton, Wembley, Middlesex.)



The new 137 Infracord spectrophotometer.



Differential Polarographic Analyser

The Nashton differential polarographic analyser is a new high sensitivity instrument, devised and manufactured in Britain for use in many different forms of nuclear, medical, industrial and agricultural research.

Metallic impurities in foodstuffs can be identified at concentrations down to 0·02 parts per million. The instrument can be used for soil analysis, for studying the oxygen content of blood plasma or for investigating respiration and photosynthesis in living cells. Many kinds of inorganic and some organic analyses can be carried out with greater accuracy than was formerly possible.
(Manufacturers: Nash & Thompson Ltd., Hook Rise South, Tolworth, Surbiton, Surrey.)

NEWS

General

Pfizer Expand Virus Research Laboratories

The latest aid to vaccine research by the Pfizer Group at Sandwich is the provision of a new specially designed building for research into virus diseases. It has extensive laboratory and animal accommodation and is designed to preclude the possibility of infectious organisms escaping to the outside as well as preventing any contamination entering the building.

The unit consists of three sections; a group of laboratories and two groups of animal blocks of four wards each, all completely separate and designed so that movement of personnel and air between each is controlled. Each animal ward and laboratory has its own high intensity ultra-violet lock entrance.

Sterile air is supplied to individual rooms and temperatures can be adjusted individually.

Personnel entering the building pass through changing and shower rooms where they change into sterile clothing. Thereafter within the building, facilities exist for further changing of clothes when entering animal wards, green overalls with hood and goggles being provided at all points. When staff leave the building they must shower before changing from their sterile clothing to their normal dress.

A preparation area and large autoclave are installed for sterilizing material entering the building. Sterile air is supplied by a large Preciptron air filter.

Solid waste leaving the building is incinerated as also is the exhaust air: liquid waste is rendered harmless before leaving the area.

One of the most important research projects to take place in this building will be the investigation of Trachoma, a virus disease which causes blindness in Africa and Asia. The Pfizer Group are co-operating with the British Medical Research Council's Trachoma Research Group in this project.

German Subsidiary for Quickfit & Quartz

Quickfit & Quartz Ltd. have formed a German subsidiary company, Quickfit Laborglas GmbH.

The new company will sell Quickfit scientific glassware in the German market. It will have its headquarters at Wiesbaden under the management of Herr Ernst Fritzsch.

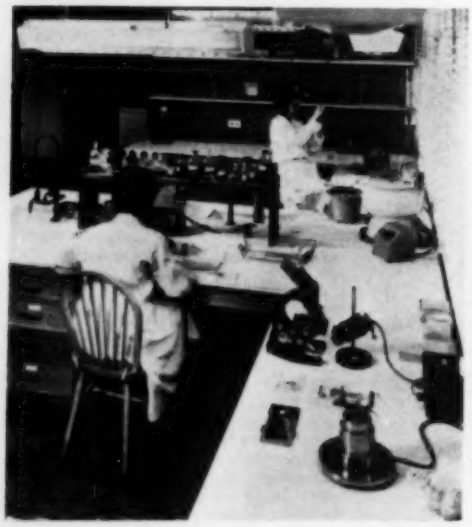
Manufacturer's News

New Equipment Company

A new company under the name of J & S Laboratory Development has been formed by A. Jolly and R. W. de Vere Stacpoole, with offices at Victoria Road, Wargrave, Berks.

Mr. Jolly is a design engineer and instrument maker and Mr. de Vere Stacpoole is a metallurgical chemist, both with many years experience in research laboratories and it was felt that there was a need for a firm to make apparatus and if necessary design it to customers' specific requirements.

There are many scientific workers in research and works laboratories who, from time to time, require the services that are available in the Universities for the design and construction of special apparatus or the modification of standard equipment and this new company will specialize in mechanical or electro-mechanical precision movements, small mechanical testing equipment, heating and humidity control, vacuum equipment and accessories. They are also interested in the development of equipment for dust sampling in view of the provisions of the Clean Air Bill.



Polar White Formica decorative laminate has been chosen for all strategic working surfaces in this section of the new pathology laboratory at St. George's Hospital (formerly called the Grove Hospital) at Tooting, London.

Change of Name

Radio Heaters Ltd. have now changed their name to Radyne Ltd.

Change of Address

Owing to the continual growth and expansion which has taken place during the post-war years, C. F. Casella & Co., Ltd., have now outgrown their premises at Regent House, Fitzroy Square, London, W.1, and have moved to Regent House, Britannia Walk, N.1.

Personal

Mr. A. H. Campbell

Hilger & Watts Ltd. announce that Mr. A. H. Campbell, M.A., M.I.E.E., who has been a director of the company since 1951 and General Manager since 1954, has been appointed Joint Managing Director with Mr. G. A. Whipple, M.A., M.I.E.E., F.Inst.P.

Mr. Campbell was a scholar at Winchester College, and Rex Moir Prizeman at Cambridge University. After practical experience in the industry with the Cambridge Instrument Company, he saw service in the last war with the Royal Corps of Signals, finally as Lt.-Col. commanding 8 Corps Signals.

For several years Mr. Campbell was Chairman of the North London District Advisory Committee for Industry, and since 1960 he has been Chairman of Council, Management Research Groups.

Mr. L. A. Thomas

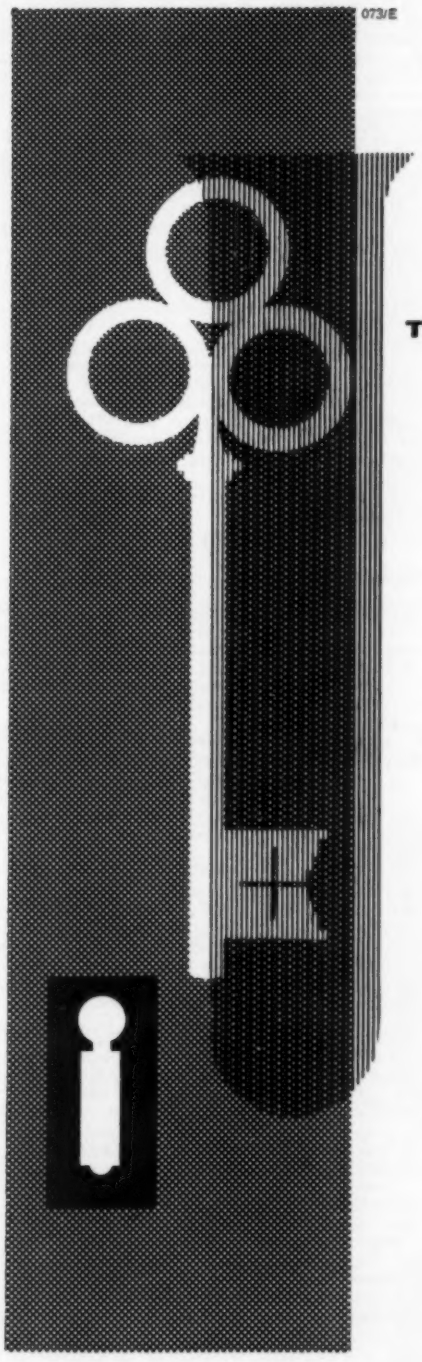
Mr. L. A. Thomas, B.Sc., F.Inst.P., A.M.I.E.E., has been appointed Chief Physicist at the Hirst Research Centre of The General Electric Co. Ltd., Wembley, Middlesex. He will be a member of the Staff of the Central Research Laboratories with special interest in the programmes of long term and fundamental research.

Mr. Thomas, who is 44 years old, joined the Research Laboratories of the G.E.C. in 1935. He was appointed Head of the Materials and Components Division in 1960 and retains his responsibilities in this field of the work.

Mr. J. E. C. Bailey

Mr. J. E. C. Bailey, C.B.E., M.I.Ex., has been appointed to the Board of Derbyshire Stone Ltd.

Mr. Bailey will continue his executive duties in the Baird & Tatlock Group, as Chairman and Managing Director of each of the operating companies in that Group, comprising Baird & Tatlock (London) Ltd., Hopkin & Williams Ltd., and W. B. Nicolson (Scientific Instruments) Ltd., all members of the Derbyshire Stone Group of Companies.



The key to success

must, first of all, fit the lock. Key and lock form an integral part and nobody will ever try to unlock a safety-lock with the church-door key.
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Mr. F. G. Paddle

Mr. F. G. Paddle, B.Sc., A.R.C.S., until recently head of the Industrial Sales Division at May & Baker Ltd., has been appointed an Assistant Director of Sales to the company. Home and Export Sales Divisions for industrial, laboratory and aromatics groups of chemicals and products now all come within his new responsibilities.

Publications

Nuclear Energy Industry

The United Kingdom Atomic Energy Authority have issued the second edition of their booklet 'The Nuclear Energy Industry of the United Kingdom.' Details are included in this edition of the wide range of chemical process plants, associated with the production and uses of nuclear power, which are now obtainable from British industry. Related chemical engineering design and consultancy services are offered to suit individual purchaser's requirements. Three new research reactors marketed by British firms are described, universities and technical colleges at which courses in nuclear science are available are listed and details of some major changes in the Authority s organization are given.

Measuring Quality in Chemical Production

In 'Quality Measurement in the Chemical Factory' (published by the Department of Scientific and Industrial Research) a physicist and a physical chemist discuss the use of instruments to control processes in the production of chemicals.

The authors sum up their point of view with the words: 'All chemical plants should have skilled analysts for measuring product quality, and it is indisputable that a first-class analyst is far more versatile than any instrument. However, if it is found that the successful functioning of a process depends on a large number of routine analytical operations, it is worth considering whether an instrument would be a more effective way of doing the job.'

The advantage of such an arrangement, they continue, is that the analyst can be released for more creative work, and that continuous control can be exercised without the delay inherent in human supervision. In this booklet, the emphasis is on instruments of proved worth and relative cheapness, and as wide a range of applications as possible.

The authors pay special attention to the use of gas chromatography, a revolutionary technique invented in Britain. Process-stream gas chromatography analysers with a simple repetition time of one minute are now becoming available, and, linked to automatic controllers, are already controlling chemical processes.

CATALOGUES, BROCHURES & LEAFLETS

Received from Manufacturers

Pyrex Glass.—The latest issue of the technical bulletin Pyrex Glass issued bi-monthly by James A. Jobling & Co., Ltd., of Wear Glass Works, Sunderland, describes the use of sintered glass filters in the more usual applications and gives details of the manufacturing process.

The Chromoscan.—This is a recording and integrating densitometer for the accurate and automatic measurement of coloured material on filter paper, start, agar, potato and cyano gels, cellulose acetate and other supporting media and for solution techniques. A leaflet describing this has been issued by Joyce, Loebl & Co., Ltd., of A8, Princesway, Team Valley, Gateshead on Tyne 11.

Pinch Valves.—The Williamson Manufacturing Co., Ltd., of Hawthorn Road, London, N.W.10, have issued a leaflet describing their Nip and Nipper pinch valves. The first of these is an all-plastic type that controls fluids at pressures up to 100 lb. per sq. in. passing through flexible tubing (either plastic or rubber) of any diameter up to $\frac{1}{4}$ in. No part is in contact with the fluid and this permits corrosive fluid control. The Nipper is an ideal substitute for the standard laboratory clamp, and is similar to the Nip. The maximum tubing diameter is $\frac{1}{8}$ in.

B.D.H. Leaflets.—New entries in the catalogue of British Drug Houses, Ltd., Poole, Dorset, are listed in the May 1961 issue of their monthly leaflet. These are: *Organic and Inorganic Chemicals*—Lead tetra-acetate; magnesium nitrate; trifluoro-acetaldehyde hydrate; trifluoro-acetamide; trifluoro-acetic acid; trifluoro-acetic anhydride. The June issue lists the following: *Organic and Inorganic Chemicals*—Acetic acid glacial aldehyde free; chloro-methyl cyanide; *p*-fluoro-nitro-benzene; *p*-fluoro-phenol; glycollonitrile 70 per cent w/w in water; *l*-naphthyl disodium orthophosphate. *Biochemicals*—hyaluronic acid potassium salt.

Scientific News.—The latest issue of 'Scientific' News published by Scientific Supplies Co., Ltd., of Scientific House, Vine Hill, London, E.C.1, contains details of burette holders, the Voss centrifuge, the new Quickfit catalogue, Speedry marking devices, SS laboratory furniture, disposable gloves and petri dishes, the Voss flame-proof stirrer, the X-Lon magnetic stirrer and the Exelo double action piston pipette.

Testing Equipment.—This is the title of a new booklet published by Clockhouse Engineering Ltd., of Brookhill Road, New Barnet, Herts. It contains illustrated descriptions of compression testing machines, proving rings and a full range of soil testing equipment, including some newly introduced items.

Boro' Price List.—Boro' Laboratories & Appliance Co., Ltd., of 1 Station Buildings, Catford Bridge, London, S.E.6, have issued a new price list (June, 1961). This contains details of all the items supplied by the company who can also quote for such products as Pyrex glassware, Monax glassware, Firmasil and Davisil glassware, E-Mil brand glassware and plastic laboratory ware.

Organic Chemical Reagents.—Monograph 42—Curcumin, Reagent for Boron, by Eileen A. Johnson, B.Sc., F.R.I.C., and W. Jablonski—has just been published by Hopkin & Williams Ltd., of Chadwell Heath, Essex.

Laboratory Furnishings.—Camlab (Glass) Ltd., of Milton Road, Cambridge, have issued the second edition of their catalogue, a substantial part of which has been devoted to both new and capital equipment.

Fluorescence Microscopy.—The latest edition of *Gurr's Commentary* published by George T. Gurr Ltd., 136-140 New Kings Road, London, S.W.6, contains notes on fluorescence microscopy with fluorochromes, all of which have been abstracted from published or communicated works.

Sales and Service Overseas.—Negretti & Zambra Ltd., of 122 Regent Street, London, W.1, have issued a catalogue outlining their connections, sales and service facilities, availability of consumable items and replacement stocks, together with addresses, etc., to enable their clients to contact the nearest source of help or information for their overseas markets.

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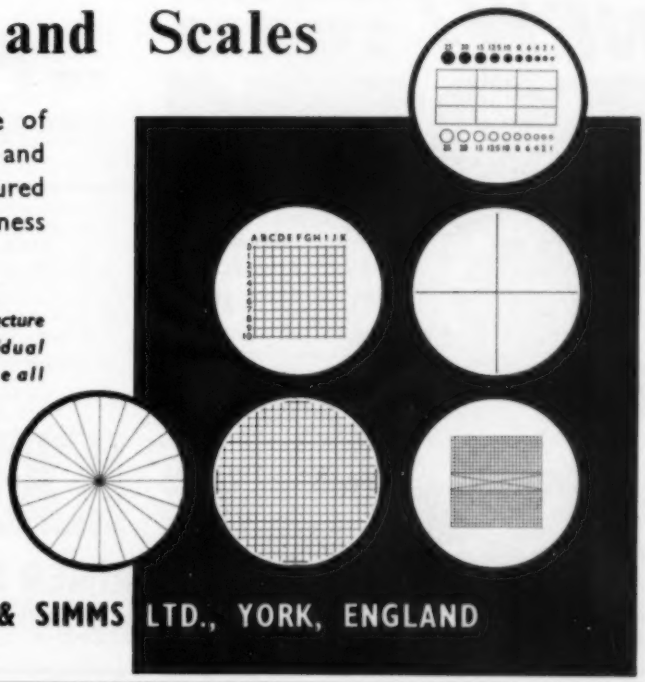

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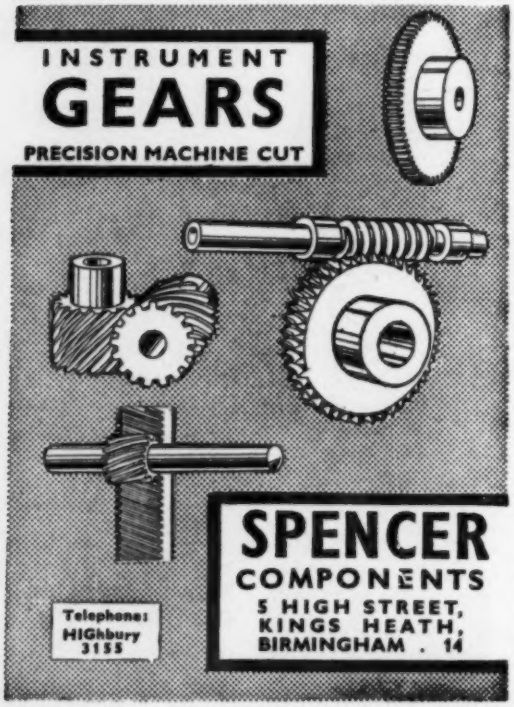

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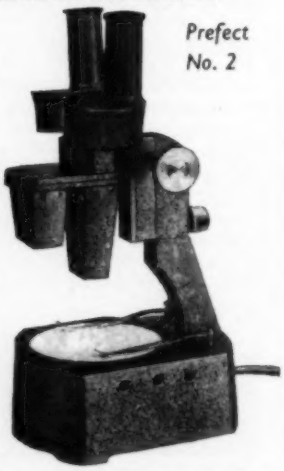

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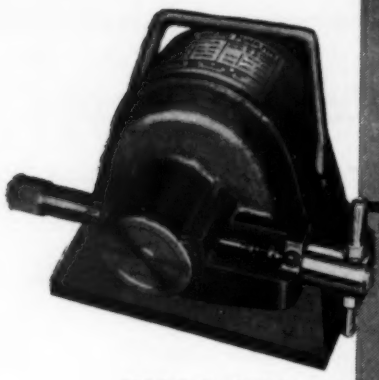

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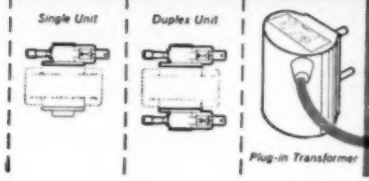

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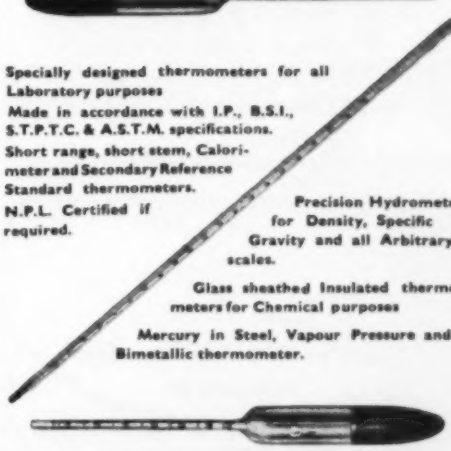

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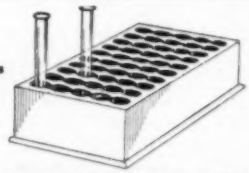

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